Claims

[Claim 1]

A method for proliferation of pancreatic progenitor cells, comprising subjecting the pancreatic progenitor cells to the following step (1):

(1) culturing the pancreatic progenitor cells in a medium containing (i) an FGF signal transduction activator, (ii) a ROCK inhibitor and (iii) a Wnt signal inhibitor,

wherein the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), and FGF3 to FGF23, and

the ROCK inhibitor is selected from N-(4-pyridinyl)-4β-[(R)-1-aminoethyl]cyclohexane-1α-carboxamide, Fasudil, (2S)-2-methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]hexahydro-1H-1,4-diazepine, 4β-[(1R)-1-aminoethyl]-N-(4-pyridyl)benzene-1α-carboxamide, N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4PER(R)-1-aminoethyl]cyclohexane-1α-carboxamide, 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, and N-(6-fluoro-1H-imidazol-5-yl)-6-methyl-2-oxo-4-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1H-pyridine-5-carboxamide; antibodies (including functional fragments), antisense nucleic acids, and siRNAs against ROCK; and antagonists of ROCK; and

the Wnt signal inhibitor is selected from IWP2, IWP3, IWP4, 2-(4-trifluoromethylphenyl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4(3H)-one, IWR1, G-CSF, IGFBP4, Dkk1, Cerberus, anti-Wnt antibodies, Wnt receptor inhibitors, and soluble Wnt receptor proteins (Frzb-1).

[Claim 2]

The proliferation method according to claim 1, wherein the pancreatic progenitor cells are pancreatic progenitor cells induced by culturing PDX1-positive cells in a medium containing (a) an EGF signal transduction activator and/or an FGF signal transduction activator and (b) a Wnt signal inhibitor,

wherein the EGF signal transduction activator is selected from EGF, TGFα, HB-EGF, Amphiregulin, Betacellulin, and Epiregulin,

the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), and FGF3 to FGF23, and

the Wnt signal inhibitor is selected from IWP2, IWP3, IWP4, 2-(4-trifluoromethylphenyl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4(3H)-one, IWR1, G-CSF, IGFBP4, Dkk1, Cerberus, anti-Wnt antibodies, Wnt receptor inhibitors, and soluble Wnt receptor proteins (Frzb-1).

[Claim 3]

A reagent for proliferation of pancreatic progenitor cells, comprising (i) an FGF signal transduction activator, (ii) a ROCK inhibitor and (iii) a Wnt signal inhibitor,

wherein the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), and FGF3 to FGF23, and

the ROCK inhibitor is selected from N-(4-pyridinyl)-4β-[(R)-1-aminoethyl]cyclohexane-1α-carboxamide, Fasudil, (2S)-2-methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]hexahydro-1H-1,4-diazepine, 4β-[(1R)-1-aminoethyl]-N-(4-pyridyl)benzene-1α-carboxamide, N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4PER(R)-1-aminoethyl]cyclohexane-1α-carboxamide, 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, and N-(6-fluoro-1H-imidazol-5-yl)-6-methyl-2-oxo-4-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1H-pyridine-5-carboxamide; antibodies (including functional fragments), antisense nucleic acids, and siRNAs against ROCK; and antagonists of ROCK; and

the Wnt signal inhibitor is selected from IWP2, IWP3, IWP4, 2-(4-trifluoromethylphenyl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4(3H)-one, IWR1, G-CSF, IGFBP4, Dkk1, Cerberus, anti-Wnt antibodies, Wnt receptor inhibitors, and soluble Wnt receptor proteins (Frzb-1).

[Claim 4]

A kit when used in proliferation of pancreatic progenitor cells, comprising (i) an FGF signal transduction activator, (ii) a ROCK inhibitor and (iii) a Wnt signal inhibitor,

wherein the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), and FGF3 to FGF23, and

the ROCK inhibitor is selected from N-(4-pyridinyl)-4β-[(R)-1-aminoethyl]cyclohexane-1α-carboxamide, Fasudil, (2S)-2-methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]hexahydro-1H-1,4-diazepine, 4β-[(1R)-1-aminoethyl]-N-(4-pyridyl)benzene-1α-carboxamide, N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4PER(R)-1-aminoethyl]cyclohexane-1α-carboxamide, 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, and N-(6-fluoro-1H-imidazol-5-yl)-6-methyl-2-oxo-4-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1H-pyridine-5-

carboxamide; antibodies (including functional fragments), antisense nucleic acids, and siRNAs against ROCK; and antagonists of ROCK; and

the Wnt signal inhibitor is selected from IWP2, IWP3, IWP4, 2-(4-trifluoromethylphenyl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4(3H)-one, IWR1, G-CSF, IGFBP4, Dkk1, Cerberus, anti-Wnt antibodies, Wnt receptor inhibitors, and soluble Wnt receptor proteins (Frzb-1).

[Claim 5]

Use of (i) an FGF signal transduction activator, (ii) a ROCK inhibitor and (iii) a Wnt signal inhibitor for proliferating pancreatic progenitor cells,

wherein the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), and FGF3 to FGF23, and

the ROCK inhibitor is selected from N-(4-pyridinyl)-4β-[(R)-1-aminoethyl]cyclohexane-1α-carboxamide, Fasudil, (2S)-2-methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]hexahydro-1H-1,4-diazepine, 4β-[(1R)-1-aminoethyl]-N-(4-pyridyl)benzene-1α-carboxamide, N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4PER(R)-1-aminoethyl]cyclohexane-1α-carboxamide, 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, and N-(6-fluoro-1H-imidazol-5-yl)-6-methyl-2-oxo-4-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1H-pyridine-5-carboxamide; antibodies (including functional fragments), antisense nucleic acids, and siRNAs against ROCK; and antagonists of ROCK; and

the Wnt signal inhibitor is selected from IWP2, IWP3, IWP4, 2-(4-trifluoromethylphenyl)-7,8-dihydro-5H-

thiopyrano[4,3-d]pyrimidin-4(3H)-one, IWR1, G-CSF, IGFBP4, Dkk1, Cerberus, anti-Wnt antibodies, Wnt receptor inhibitors, and soluble Wnt receptor proteins (Frzb-1).

[Claim 6]

A method for production of pancreatic progenitor cells, comprising the steps of: culturing and proliferating pancreatic progenitor cells in a medium containing (i) an FGF signal transduction activator, (ii) a ROCK inhibitor and (iii) a Wnt signal inhibitor; and collecting the pancreatic progenitor cells from the cultures,

wherein the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), and FGF3 to FGF23, and

the ROCK inhibitor is selected from N-(4-pyridinyl)-4β-[(R)-1-aminoethyl]cyclohexane-1α-carboxamide, Fasudil, (2S)-2-methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]hexahydro-1H-1,4-diazepine, 4β-[(1R)-1-aminoethyl]-N-(4-pyridyl)benzene-1α-carboxamide, N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4PER(R)-1-aminoethyl]cyclohexane-1α-carboxamide, 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, and N-(6-fluoro-1H-imidazol-5-yl)-6-methyl-2-oxo-4-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1H-pyridine-5-carboxamide; antibodies (including functional fragments), antisense nucleic acids, and siRNAs against ROCK; and antagonists of ROCK; and

the Wnt signal inhibitor is selected from IWP2, IWP3, IWP4, 2-(4-trifluoromethylphenyl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4(3H)-one, IWR1, G-CSF, IGFBP4,

Dkk1, Cerberus, anti-Wnt antibodies, Wnt receptor inhibitors, and soluble Wnt receptor proteins (Frzb-1).

[Claim 7]

A method for production of pancreatic progenitor cells, comprising the steps of: culturing PDX1-positive cells in a medium containing (a) an EGF signal transduction activator and/or an FGF signal transduction activator and (b) a Wnt signal inhibitor to induce differentiation thereof into the pancreatic progenitor cells; and then

culturing and proliferating pancreatic progenitor cells in a medium containing (i) an FGF signal transduction activator, (ii) a ROCK inhibitor and (iii) a Wnt signal inhibitor; and collecting the pancreatic progenitor cells from the cultures,

wherein the EGF signal transduction activator is selected from EGF, TGFα, HB-EGF, Amphiregulin, Betacellulin, and Epiregulin,

the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), and FGF3 to FGF23, and

the ROCK inhibitor is selected from N-(4-pyridinyl)-4β-[(R)-1-aminoethyl]cyclohexane-1α-carboxamide, Fasudil, (2S)-2-methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]hexahydro-1H-1,4-diazepine, 4β-[(1R)-1-aminoethyl]-N-(4-pyridyl)benzene-1α-carboxamide, N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4PER(R)-1-aminoethyl]cyclohexane-1α-carboxamide, 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, and N-(6-fluoro-1H-imidazol-5-yl)-6-methyl-2-oxo-4-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1H-pyridine-5-carboxamide; antibodies (including functional fragments),

antisense nucleic acids, and siRNAs against ROCK; and antagonists of ROCK; and

the Wnt signal inhibitor is selected from IWP2, IWP3, IWP4, 2-(4-trifluoromethylphenyl)-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidin-4(3H)-one, IWR1, G-CSF, IGFBP4, Dkk1, Cerberus, anti-Wnt antibodies, Wnt receptor inhibitors, and soluble Wnt receptor proteins (Frzb-1).