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), FGF3 to FGF23, and their functional analogs, 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; antagonists and dominant negative forms of ROCK; and other ROCK inhibitors disclosed in US2005-0209261, US2005-0192304, US2004-0014755, US2004-0002508, US2004-0002507, US2003-0125344, WO2003/082808, US2003-0087919, WO2005/035506, WO2005/074643, WO2004/039796, WO2003/062227, WO2003/062225, WO2003/059913, WO2002/076976, WO2002/076977, WO01/17562, WO00/78351, and WO98/06433, 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 agonists (Wnt receptor inhibitors), soluble Wnt receptor proteins (Frzb-1), and dominant negative forms.

[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, functional analogs thereof, TGFα, HB-EGF, Amphiregulin, Betacellulin, and Epiregulin,

the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), FGF3 to FGF23, and their functional analogs, 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 agonists (Wnt receptor inhibitors), soluble Wnt receptor proteins (Frzb-1), and dominant negative forms.

[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), FGF3 to FGF23, and their functional analogs, 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; antagonists and dominant negative forms of ROCK; and other ROCK inhibitors disclosed in US2005-0209261, US2005-0192304, US2004-0014755, US2004-0002508, US2004-0002507, US2003-0125344, WO2003/082808, US2003-0087919, WO2005/035506, WO2005/074643, WO2004/039796, WO2003/062227, WO2003/062225, WO2003/059913, WO2002/076976, WO2002/076977, WO01/17562, WO00/78351, and WO98/06433, 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 agonists (Wnt receptor inhibitors), soluble Wnt receptor proteins (Frzb-1), and dominant negative forms.

[Claim 4]

A kit 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), FGF3 to FGF23, and their functional analogs, 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; antagonists and dominant negative forms of ROCK; and other ROCK inhibitors disclosed in US2005-0209261, US2005-0192304, US2004-0014755, US2004-0002508, US2004-0002507, US2003-0125344, WO2003/082808, US2003-0087919, WO2005/035506, WO2005/074643, WO2004/039796, WO2003/062227, WO2003/062225, WO2003/059913, WO2002/076976, WO2002/076977, WO01/17562, WO00/78351, and WO98/06433, 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 agonists (Wnt

receptor inhibitors), soluble Wnt receptor proteins (Frzb-1), and dominant negative forms.

[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), FGF3 to FGF23, and their functional analogs, 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; antagonists and dominant negative forms of ROCK; and other ROCK inhibitors disclosed in US2005-0209261, US2005-0192304, US2004-0014755, US2004-0002508, US2004-0002507, US2003-0125344, WO2003/082808, US2003-0087919, WO2005/035506, WO2005/074643, WO2004/039796, WO2003/062227, WO2003/062225, WO2003/059913, WO2002/076976, WO2002/076977, WO01/17562, WO00/78351, and WO98/06433, 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 agonists (Wnt receptor inhibitors), soluble Wnt receptor proteins (Frzb-1), and dominant negative forms.

[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), FGF3 to FGF23, and their functional analogs, 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; antagonists and dominant negative forms of ROCK; and other ROCK inhibitors disclosed in US2005-0209261, US2005-0192304, US2004-0014755, US2004-0002508, US2004-0002507, US2003-0125344, WO2003/082808, US2003-0087919, WO2005/035506, WO2005/074643, WO2004/039796, WO2003/062227,

WO2003/062225, WO2003/059913, WO2002/076976, WO2002/076977, WO01/17562, WO00/78351, and WO98/06433, 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 agonists (Wnt receptor inhibitors), soluble Wnt receptor proteins (Frzb-1), and dominant negative forms.

[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, functional analogs thereof, TGFα, HB-EGF, Amphiregulin, Betacellulin, and Epiregulin,

the FGF signal transduction activator is selected from aFGF (FGF1), bFGF (FGF2), FGF3 to FGF23, and their functional analogs, 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; antagonists and dominant negative forms of ROCK; and other ROCK inhibitors disclosed in US2005-0209261, US2005-0192304, US2004-0014755, US2004-0002508, US2004-0002507, US2003-0125344, WO2003/082808, US2003-0087919, WO2005/035506, WO2005/074643, WO2004/039796, WO2003/062227, WO2003/062225, WO2003/059913, WO2002/076976, WO2002/076977, WO01/17562, WO00/78351, and WO98/06433, 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 agonists (Wnt receptor inhibitors), soluble Wnt receptor proteins (Frzb-1), and dominant negative forms.