

WHAT IS CLAIMED IS:

1. A method of producing splanchnic mesoderm cells, comprising:
contacting lateral plate mesoderm cells with a TGF-beta signaling pathway inhibitor, a Wnt signaling pathway inhibitor, a BMP signaling pathway activator, an FGF signaling pathway activator, and a retinoic acid (RA) signaling pathway activator for a period of time sufficient to differentiate lateral plate mesoderm cells to splanchnic mesoderm cells.
2. The method of claim 1, wherein the splanchnic mesoderm cells are human splanchnic mesoderm cells.
3. The method of claim 1 or -2, wherein the lateral plate mesoderm cells have been differentiated from middle primitive stream cells.
4. The method of claim 3, wherein the lateral plate mesoderm cells have been differentiated from middle primitive streak cells by contacting the middle primitive streak cells with a TGF-beta signaling pathway inhibitor, a Wnt signaling pathway inhibitor, and a BMP signaling pathway activator.
5. The method of claim 3 or 4, wherein the middle primitive streak cells have been differentiated from pluripotent stem cells.
6. The method of claim 5, wherein the middle primitive streak cells have been differentiated from pluripotent stem cells by contacting the pluripotent stem cells with a TGF-beta signaling pathway activator, a Wnt signaling pathway activator, an FGF signaling pathway activator, a BMP signaling pathway activator, and a PI3K signaling pathway inhibitor.
7. The method of any one of claims 1 to -6, wherein the lateral plate mesoderm cells are contacted with A8301, BMP4, C59, FGF2, RA, or any combination thereof.
8. The method of any one of claims 1 to -7, wherein ~~the lateral plate mesoderm cells are contacted for a period of time~~ time that is sufficient to differentiate lateral plate mesoderm cells to splanchnic mesoderm cells, and/or for a time that is or is about 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 hours, or any time within a range defined by any two of the aforementioned times.

~~9. — The method of any one of claims 1-8, wherein the lateral plate mesoderm cells are contacted for a time that is or is about 48 hours.~~

~~10.9. The method of any one of claims 1 to -89, wherein the splanchnic mesoderm cells exhibit increased expression of FOXF1, HOXA1, HOXA5, or WNT2, or any combination thereof, and decreased expression of NKX2-5, ISL1, or TBX2, or any combination thereof, relative to cardiac mesoderm cells.~~

~~11. — The method of any one of claims 1-10, wherein the splanchnic mesoderm cells exhibit decreased expression of PAX3 or PRRX1, or both, relative to middle primitive streak cells, and/or decreased expression of CD31 relative to cardiac mesoderm cells.~~

~~12.10. A method of producing septum transversum cells, comprising contacting splanchnic mesoderm cells with a retinoic acid signaling pathway activator and a BMP signaling pathway activator for a period of time sufficient to differentiate splanchnic mesoderm cells to septum transversum cells.~~

~~13.11. The method of claim 12.10, wherein the splanchnic mesoderm cells are the splanchnic mesoderm cells produced by the method of any one of claims 1 to -119.~~

~~14.12. The method of claim 12.10 or 13.11, wherein the splanchnic mesoderm cells are contacted with RA, BMP4, or both.~~

~~15.13. The method of any one of claims 12-14.10 to 12, wherein the splanchnic mesoderm cells are contacted for a period of time that is sufficient to differentiate splanchnic mesoderm cells to septum transversum cells, and/or for a time that is or is about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, or -84 hours, or any period of time within a range defined by any two of the aforementioned times.~~

~~16. — The method of any one of claims 12-15, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 72 hours.~~

~~17. — The method of any one of claims 12-16, wherein the septum transversum cells exhibit increased expression of WT1, TBX18, LHX2, UPK3B, or UPK1B, or any combination~~

thereof, relative to cardiac mesoderm cells, splanchnic mesoderm cells, or fibroblasts, or any combination thereof.

18. — The method of any one of claims 12-17, wherein the septum transversum cells exhibit decreased expression of MSX1, MSX2, or HAND1, or any combination thereof, relative to cardiac mesoderm cells or fibroblasts, or both.

19. — The method of any one of claims 12-18, wherein the septum transversum cells exhibit decreased expression of HOXA1 or TBX5, or both, relative to splanchnic mesoderm cells.

20. — The method of any one of claims 12-19, wherein the septum transversum cells exhibit decreased expression of NKX6.1 or HOXA5, or both, relative to respiratory mesenchyme cells.

21. — The method of any one of claims 12-20, wherein the septum transversum cells exhibit decreased expression of NKX3.2, MSC, BARX1, WNT4, or HOXA5, or any combination thereof, relative to esophageal/gastric mesenchyme cells.

22. — The method of any one of claims 12-21, wherein the septum transversum cells account for about 60%, 65%, 70%, 75%, 80%, 85%, or 90% of the total cells differentiated from the splanchnic mesoderm cells.

23. — A method of producing fibroblasts, comprising contacting splanchnic mesoderm cells with a retinoic acid signaling pathway activator, a BMP signaling pathway activator, and a Wnt signaling pathway activator.

24. — The method of claim 23, wherein the splanchnic mesoderm cells are the splanchnic mesoderm cells of any one of claims 1-11.

25. — The method of claim 23 or 24, wherein the splanchnic mesoderm cells are contacted with RA, BMP4, CHIR99021, or any combination thereof.

26. — The method of any one of claims 23-25, wherein the fibroblasts are liver fibroblasts.

~~27. The method of any one of claims 23-26, wherein the splanchnic mesoderm cells are contacted for a time that is sufficient to differentiate splanchnic mesoderm cells to fibroblasts, and/or for a time that is or is about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, or 84 hours, or any period of time within a range defined by any two of the aforementioned times.~~

~~28. The method of any one of claims 23-27, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 72 hours.~~

~~29. The method of any one of claims 23-28, wherein the fibroblasts exhibit increased expression of MSX1, MSX2, or HAND1, or any combination thereof, relative to splanchnic mesoderm cells or septum transversum cells, or both.~~

~~30. The method of any one of claims 23-29, wherein the fibroblasts exhibit decreased expression of WT1, TBX18, LHX2, or UPK1B, or any combination thereof, relative to septum transversum cells.~~

~~31. The method of any one of claims 23-30, wherein the fibroblasts exhibit decreased expression of NKX6.1, HOXA5, or LHX2, or any combination thereof, relative to respiratory mesenchyme cells.~~

~~32.14. The method of any one of claims 23-31, wherein the fibroblasts exhibit decreased expression of NKX3.2, MSC, BARX1, WNT4, or HOXA5, or any combination thereof, relative to esophageal/gastric mesenchyme cells.~~

33.15. A method of producing respiratory mesenchyme cells, comprising a) contacting splanchnic mesoderm cells with a retinoic acid signaling pathway activator, a BMP signaling pathway activator, a hedgehog (HH) signaling pathway activator, and a Wnt signaling pathway activator for a period of time sufficient to differentiate splanchnic mesoderm cells to respiratory mesenchyme cells.

34.16. The method of claim 33, wherein the splanchnic mesoderm cells are contacted for a period of time that is sufficient to differentiate splanchnic mesoderm cells to respiratory mesenchyme cells, and/or for a time that is or is about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69,

~~70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, or 84 hours, or any period of time within a range defined by any two of the aforementioned times.~~

~~35. The method of claim 33 or 34, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 72 hours.~~

~~36.17. The method of claim 3315 or 16, wherein step a) is a second step, and further comprising a first step of contacting the splanchnic mesoderm cells with a retinoic acid signaling pathway activator, a BMP signaling pathway activator, and a HH signaling pathway activator prior to the second step.~~

~~37. The method of claim 36, wherein the splanchnic mesoderm cells are contacted for a time that is sufficient to differentiate splanchnic mesoderm cells to respiratory mesenchyme cells, and/or for a time or is about 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 hours, or any period of time within a range defined by any two of the aforementioned times for the first step.~~

~~38. The method of claim 36 or 37, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 48 hours for the first step.~~

~~39. The method of any one of claims 36-38, wherein the splanchnic mesoderm cells are contacted for a time that is sufficient to differentiate splanchnic mesoderm cells to respiratory mesenchyme cells, and/or for a time or is about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 hours, or any period of time within a range defined by any two of the aforementioned times for the second step.~~

~~40. The method of any one of claims 36-39, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 24 hours for the second step.~~

~~41.18. The method of any one of claims 33-4015 to 17, wherein the splanchnic mesoderm cells are the splanchnic mesoderm cells produced by the method of any one of claims 1 to 9-11.~~

~~42.19. The method of any one of claims 33-4115 to 18, wherein the splanchnic mesoderm cells are contacted with RA, BMP4, PMA, CHIR99021, or any combination thereof.~~

43. — ~~The method of any one of claims 33-42, wherein the respiratory mesenchyme cells exhibit increased expression of NKX6-1, TBX5, HOXA1, HOXA5, FOXF1, LHX2, or WNT2, or any combination thereof, relative to cardiac endoderm cells, splanchnic mesoderm cells, or esophageal/gastric mesenchyme cells, or any combination thereof.~~

44. — ~~The method of any one of claims 33-43, wherein the respiratory mesenchyme cells exhibit decreased expression of WNT2, WT1, TBX18, LHX2, or UPK1B, or any combination thereof, relative to septum transversum cells.~~

45. — ~~The method of any one of claims 33-44, wherein the respiratory mesenchyme cells exhibit decreased expression of WNT2, MSX1, or MSX2, or any combination thereof, relative to fibroblast cells.~~

46.20. A method of producing esophageal/gastric mesenchyme cells, comprising a) contacting splanchnic mesoderm cells with a retinoic acid signaling pathway activator, a BMP signaling pathway inhibitor, and a HH signaling pathway activator.

47. — ~~The method of claim 46, wherein the splanchnic mesoderm cells are contacted for a time that is sufficient to differentiate splanchnic mesoderm cells to esophageal/gastric mesenchyme cells, and/or for a time or is about 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, or 84 hours, or any period of time within a range defined by any two of the aforementioned times.~~

48. — ~~The method of claim 46 or 47, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 72 hours.~~

49. — ~~The method of claim 46, wherein step a) is a second step, and further comprising a first step of contacting the splanchnic mesoderm cells with a retinoic acid signaling pathway activator and a HH signaling pathway activator prior to the second step.~~

50. — ~~The method of claim 49, wherein the splanchnic mesoderm cells are contacted for a time that is sufficient to differentiate splanchnic mesoderm cells to esophageal/gastric mesenchyme cells, and/or for a time or is about 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47,~~

~~48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 hours, or any period of time within a range defined by any two of the aforementioned times for the first step.~~

~~51.—The method of claim 49 or 50, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 48 hours for the first step.~~

~~52.—The method of any one of claims 49–51, wherein the splanchnic mesoderm cells are contacted for a time that is sufficient to differentiate splanchnic mesoderm cells to esophageal/gastric mesenchyme cells, and/or for a time or is about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 hours, or any period of time within a range defined by any two of the aforementioned times for the second step.~~

~~53.—The method of any one of claims 49–52, wherein the splanchnic mesoderm cells are contacted for a period of time that is or is about 24 hours for the second step.~~

~~54.—The method of claim 46–53, wherein the splanchnic mesoderm cells are the splanchnic mesoderm cells of any one of claims 1–11.~~

~~55.—The method of any one of claims 46–54, wherein the splanchnic mesoderm cells are contacted with RA, Noggin, PMA, or any combination thereof.~~

~~56.—The method of any one of claims 46–55, wherein the esophageal/gastric mesenchyme cells exhibit increased expression of MSC, BARX1, WNT4, HOXA1, FOXF1, or NKX3-2, or any combination thereof, relative to cardiac endoderm cells, splanchnic mesoderm cells, or respiratory mesenchyme cells, or any combination thereof.~~

~~57.—The method of any one of claims 46–56, wherein the esophageal/gastric mesenchyme cells exhibit decreased expression of WNT2, TBX5, MSX1, MSX2, or LHX2, or any combination thereof, relative to septum transversum cells, fibroblasts, or respiratory mesenchyme cells, or any combination thereof.~~

~~58.—The method of any one of claims 1–57, wherein the TGF- β signaling pathway inhibitor is selected from the group consisting of A8301, RepSox, LY365947, and SB431542.~~

59. — The method of any one of claims 1-58, wherein the TGF-beta signaling pathway inhibitor is A8301.

60. — The method of any one of claims 1-59, wherein the TGF-beta signaling pathway inhibitor is contacted at a concentration of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 μ M, or about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 μ M, or any concentration within a range defined by any two of the aforementioned concentrations.

61. — The method of any one of claims 1-60, wherein the TGF-beta signaling pathway inhibitor is contacted at concentration of 1 μ M or about 1 μ M.

62. — The method of any one of claims 1-61, wherein the Wnt signaling pathway inhibitor is selected from the group consisting of C59, PNU 74654, KY-02111, PRI-724, FH-535, DIF-1, and XAV939.

63. — The method of any one of claims 1-62, wherein the Wnt signaling pathway inhibitor is C59.

64. — The method of any one of claims 1-63, wherein the Wnt signaling pathway inhibitor is contacted at a concentration of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 μ M, or about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 μ M, or any concentration within a range defined by any two of the aforementioned concentrations.

65. — The method of any one of claims 1-64, wherein the Wnt signaling pathway inhibitor is contacted at a concentration of 1 μ M or about 1 μ M.

66. — The method of any one of claims 1-65, wherein the BMP signaling pathway activator is selected from the group consisting of BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP10, BMP11, BMP15, IDE1, and IDE2.

67. — The method of any one of claims 1-66, wherein the BMP signaling pathway activator is BMP4.

~~68. The method of any one of claims 1-67, wherein the BMP signaling pathway activator is contacted at a concentration of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 ng/mL or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45 ng/mL, or any concentration within a range defined by any two of the aforementioned concentrations.~~

~~69. The method of any one of claims 1-68, wherein the BMP signaling pathway activator is contacted at a concentration of 30 ng/mL or about 30 ng/mL.~~

~~70. The method of any one of claims 1-69, wherein the FGF signaling pathway activator is selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF4, FGF5, FGF6, FGF7, FGF8, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, and FGF23.~~

~~71. The method of any one of claims 1-70, wherein the FGF signaling pathway activator is FGF2.~~

~~72. The method of any one of claims 1-71, wherein the FGF signaling pathway activator is contacted at a concentration of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 ng/mL, or about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 ng/mL, or any concentration within a range defined by any two of the aforementioned concentrations.~~

~~73. The method of any one of claims 1-72, wherein the FGF signaling pathway activator is contacted at a concentration of 20 ng/mL or about 20 ng/mL.~~

~~74. The method of any one of claims 1-73, wherein the RA signaling pathway activator is selected from the group consisting of retinoic acid, all-trans retinoic acid, 9-cis retinoic acid, CD437, EC23, BS 493, TTNPB, and AM580.~~

~~75. The method of any one of claims 1-74, wherein the RA signaling pathway activator is RA.~~

~~76.—The method of any one of claims 1-75, wherein the RA signaling pathway activator is contacted at a concentration of 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.9, or 3 μ M, or about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.9, or 3 μ M, or any concentration within a range defined by any two of the aforementioned concentrations.~~

~~77.—The method of any one of claims 1-76, wherein the RA signaling pathway activator is contacted at a concentration of 2 μ M or about 2 μ M.~~

~~78.—The method of any one of claims 1-77, wherein the Wnt signaling pathway activator is selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt10b, Wnt11, Wnt16, BML 284, IQ-1, WAY 262611, CHIR99021, CHIR 98014, AZD2858, BIO, AR-A014418, SB 216763, SB 415286, aloisine, indirubin, alsterpaullone, kenpaullone, lithium chloride, TDZD-8, and TWS119.~~

~~79.—The method of any one of claims 1-78, wherein the Wnt signaling pathway activator is CHIR99021.~~

~~80.—The method of any one of claims 1-79, wherein the Wnt signaling pathway activator is contacted at a concentration of 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 μ M, or about 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 μ M, or any concentration within a range defined by any two of the aforementioned concentrations.~~

~~81.—The method of any one of claims 1-80, wherein the Wnt signaling pathway activator is contacted at a concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μ M, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μ M, or any concentration within a range defined by any two of the aforementioned concentrations.~~

~~82.—The method of any one of claims 1-81, wherein the HH signaling pathway activator is selected from the group consisting of SHH, IHH, DHH, PMA, GSA-10, and SAG.~~

~~83. The method of any one of claims 1-82, wherein the HH signaling pathway activator is PMA.~~

~~84. The method of any one of claims 1-83, wherein the HH signaling pathway activator is contacted at a concentration of 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3 μ M or about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3 μ M, or any concentration within a range defined by any two of the aforementioned concentrations.~~

~~85. The method of any one of claims 1-84, wherein the HH signaling pathway activator is contacted at a concentration of 2 μ M or about 2 μ M.~~

~~86. The method of any one of claims 1-85, wherein the BMP signaling pathway inhibitor is selected from the group consisting of Noggin, RepSox, LY364947, LDN193189, and SB431542.~~

~~87. The method of any one of claims 1-86, wherein the BMP signaling pathway inhibitor is Noggin.~~

~~88. The method of any one of claims 1-87, wherein the BMP signaling pathway inhibitor is contacted at a concentration of 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 ng/mL or about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 ng/mL, or any concentration within a range defined by any two of the aforementioned concentrations.~~

~~89. The method of any one of claims 1-88, wherein the BMP signaling pathway inhibitor is contacted at a concentration of 100 ng/mL or about 100 ng/mL.~~

~~90.21. The An isolated splanchnic mesoderm cells produced by the method of any one of claims 1 to 9-11.~~

~~91.22. The An isolated septum transversum cells produced by the method of any one of claims 12-2210 to 13.~~

~~92.23. The An isolated fibroblasts produced by the method of any one of claims 23-3214.~~

93.24. ~~The~~ An isolated respiratory mesenchyme cells produced by the method of any one of claims ~~33-45~~ 15 to 19.

94.25. ~~The~~ An isolated esophageal/gastric mesenchyme cells produced by the method of any one of claim ~~20s~~ 46-57.