the activating and/or inhibiting of one or more signaling pathways within a precursor cell. Also disclosed are methods for using the disclosed gastric cells, gastric tissues, and or gastric organoids derived from precursor cells.

[0006a] In one embodiment, there is provided a method of inducing formation of a gastric tissue, comprising the steps of:

a) activating a combination of a WNT signaling pathway and a FGF signaling pathway within a definitive endoderm derived from an induced pluripotent stem cell, an embryonic stem cell, and/or an endoderm cell, wherein said WNT signalling pathway is activated using an agent selected from one or more molecules selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt9a, Wnt10a, Wnt10b, Wnt11, Wnt16, a GSK3β inhibitor such as Chiron, and small molecule modulators that activate the WNT signalling pathway, and wherein said FGF signalling pathway is activated using an agent selected from one or more molecules selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, FGF23 and small molecule modulators that activate the FGF signalling pathway,

b) inhibiting a BMP signaling pathway within said definitive endoderm derived from an induced pluripotent stem cell, embryonic stem cell, and/or endoderm cell by contacting said definitive endoderm derived from an induced pluripotent stem cell, embryonic stem cell, and/or endoderm cell with an agent that inhibits the BMP pathway, wherein said agent is selected from Noggin, Dorsomorphin, LDN189, DMH-1, or small molecule modulators that inhibit the BMP signaling pathway; and

c) administering retinoic acid to said pluripotent stem cell;

wherein said steps a) through c) are carried out for a period of time sufficient to cause formation of a foregut spheroid, further

comprising the step of contacting said foregut spheroid with EGF, RA, and a BMP inhibitor.

[0006b] In one embodiment, there is provided method of generating a gastric organoid comprising fundus tissue comprising the steps of

a) generating a definitive endoderm (DE) from a pluripotent stem cell;

b) contacting said definitive endoderm with an agent that activates the FGF pathway as defined herein, an agent that activates the WNT pathway as defined in herein, an agent that inhibits the BMP pathway as defined herein, and retinoic acid to generate a posterior foregut; and

c) contacting said posterior foregut with EGF, retinoic acid, an agent that activates the WNT pathway as defined herein, and an agent that inhibits the BMP pathway as defined herein, to generate a fundus tissue.

[0006c] In one embodiment, there is provided a method of generating a gastric organoid comprising antrum tissue comprising the steps of:

a) generating a definitive endoderm (DE) from a pluripotent stem cell;

b) contacting said definitive endoderm with an agent that activates the FGF pathway as defined herein, an agent that activates the WNT pathway as defined herein, an agent that inhibits the BMP pathway as defined herein, and retinoic acid to generate a posterior foregut; and

c) contacting said posterior foregut with EGF, retinoic acid, and an agent that inhibits the BMP pathway as defined herein, to generate an antrum tissue.

Brief Description of the Drawings

[0007] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0008] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application

[0009] publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0010] **FIG 1** depicts expression of Sox2/Cdx2/B-catenin in gastric spheroids and the effect of RA.

[0011] **FIG 2A-2E** depicts schematic representation of the *in vitro* culture system used to direct the differentiation of hPSCs into three-dimensional gastric organoids (FIG 2A), the defining markers of developing posterior foregut organs by wholemount immunofluorescent staining of mouse E10.5 embryos with Sox2, Pdx1 and Cdx2 (FIG 2B), PDX1 expression in the presence and absence of RA (FIG 2C), stereomicrographs showing morphological changes during growth of posterior foregut spheroids into hGOs (FIG 2D), and a comparison of developing mouse antrum at E14.5 and E18.5 and comparable stages of hGO development (FIG 2E).

[0012] **FIG 3A-3D** depictsMuc5AC, TFF2, GSII UEAI, and CHGA expression in P12 Antrum, E18.5 Antrum and d34 Organoid (FIG

CLAIMS

1. A method of inducing formation of a gastric tissue, comprising the steps of:

a) activating a combination of a WNT signaling pathway and a FGF signaling pathway within a definitive endoderm derived from an induced pluripotent stem cell, an embryonic stem cell, and/or an endoderm cell, wherein said WNT signalling pathway is activated using an agent selected from one or more molecules selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt9a, Wnt10a, Wnt10b, Wnt11, Wnt16, a GSK3β inhibitor such as Chiron, and small molecule modulators that activate the WNT signalling pathway, and wherein said FGF signalling pathway is activated using an agent selected from one or more molecules selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, FGF23 and small molecule modulators that activate the FGF signalling pathway,

b) inhibiting a BMP signaling pathway within said definitive endoderm derived from an induced pluripotent stem cell, embryonic stem cell, and/or endoderm cell by contacting said definitive endoderm derived from an induced pluripotent stem cell, embryonic stem cell, and/or endoderm cell with an agent that inhibits the BMP pathway, wherein said agent is selected from Noggin, Dorsomorphin, LDN189, DMH-1, or small molecule modulators that inhibit the BMP signaling pathway; and

c) administering retinoic acid to said pluripotent stem cell;

wherein said steps a) through c) are carried out for a period of time sufficient to cause formation of a foregut spheroid, further comprising the step of contacting said foregut spheroid with EGF, RA, and a BMP inhibitor.

2. The method of claim 1, wherein said precursor cell is selected from an embryonic stem cell, an induced pluripotent stem cell, a mesoderm cell, a definitive endoderm cell, a posterior endoderm cell, a posterior endoderm cell, and a hindgut cell, preferably a definitive endoderm derived from a pluripotent stem cell, more preferably a definitive endoderm derived from a pluripotent stem cell selected from an embryonic stem cell, an adult stem cell, or an induced pluripotent stem cell.

3. The method of any one of claims 1 or 2, wherein said precursor cell is a definitive endoderm derived from contacting a pluripotent stem cell with one or more molecules selected from Activin, the BMP subgroups of the TGF-beta superfamily of growth factors; Nodal, Activin A, Activin B, BMP4, Wnt3a, and combinations thereof.

4. The method of any one of claims 1 to 3, wherein said small molecule modulator that activate the WNT signalling pathway is selected from the group consisting of : Lithium Chloride; 2-amino-4,6-disubstituted pyrimidine (hetero) arylpyrimidines; IQ1; QS11; NSC668036; DCA beta-catenin; and 2-amino-4-[3,4-(methylenedioxy)-benzyl-amino]-6-(3-methoxyphenyl) pyrimidine.

5. The method of any one of claims 1 to 4, wherein said FGF signalling pathway is activated using FGF4, more preferably FGF4 at a concentration between about 50 to about 1500 ng/ml.

6. The method of any one of claims 1 to 5, wherein said WNT signalling pathway is activated using Wnt3a, preferably Wnt3a at a concentration between about 50 to about 1500 ng/ml.

7. The method of any one of claims 1 to 6, wherein said activating step comprises contacting said precursor cell with both Wnt3a and FGF4 or CHIR99021 and FGF4 over a specified activation period.

8. The method of any one of claims 1 to 7, wherein said precursor cell is contacted with a BMP inhibitor at a concentration between about 50 to about 1500 ng/ml.

9. The method of any one of claims 1 to 8, wherein said steps a) through c) are carried out for a period of time sufficient to cause formation of a foregut spheroid, preferably wherein steps a) and b) are carried out prior to step c).

10. The method of any one of claims 1 to 9, wherein the retinoic acid of step c) is administered in an amount of between about 0.2 uM to about 20 uM, preferably about 2.0 uM, preferably wherein step c) is carried out until expression of Pdx1/Sox1 is detected in said foregut spheroids.

11. The method of any one of claims 1 to 10, wherein said exposure to EGF is at a concentration and length of time sufficient to increase said gastric spheroids to greater than 1 mm in diameter, or greater than 2 mm in diameter, or greater than 3 mm in diameter, or greater than 4 mm in diameter.

12. The method of any one of claims 1 to 11, further comprising the step of culturing said activated precursor cell *in vitro* to form a 3-dimensional tissue structure.

13. The method of any one of claims 1 to 12, wherein said activating and inhibiting steps are conducted *in vitro*.

14. The method of any one of claims 1 to 13, wherein said activation period and said inhibition period overlap.

15. The method of any one of claims 1 to 13, wherein said activation period and said inhibition period do not overlap.

16. The method of any one of claims 1 to 15, wherein said activation period is between about 24 and about 120 hours.

17. A method of generating a gastric organoid comprising fundus tissue comprising the steps of

a) generating a definitive endoderm (DE) from a pluripotent stem cell;

b) contacting said definitive endoderm with an agent that activates the FGF pathway as defined in claim 1, an agent that activates the WNT pathway as defined in claim 1 or claim 4, an agent that inhibits the BMP pathway as defined in claim 1, and retinoic acid to generate a posterior foregut; and

c) contacting said posterior foregut with EGF, retinoic acid, an agent that activates the WNT pathway as defined in claim 1 or claim 4, and an agent that inhibits the BMP pathway as defined in claim 1, to generate a fundus tissue.

18. A method of generating a gastric organoid comprising antrum tissue comprising the steps of:

a) generating a definitive endoderm (DE) from a pluripotent stem cell;

b) contacting said definitive endoderm with an agent that activates the FGF pathway as defined in claim 1, an agent that activates the WNT pathway as defined in claim 1 or claim 4, an agent that inhibits the BMP pathway as defined in claim 1, and retinoic acid to generate a posterior foregut; and

c) contacting said posterior foregut with EGF, retinoic acid, and an agent that inhibits the BMP pathway as defined in claim 1, to generate an antrum tissue.