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 aspect provided herein is an *in vitro* method of inducing formation of a gastric tissue, comprising the steps of: a) contacting a definitive endoderm cell with a WNT signaling pathway activator, an FGF signaling pathway activator, and a BMP signaling pathway inhibitor for a period of time sufficient to form SOX2+ foregut cells; b) contacting the SOX2+ foregut cells with retinoic acid for a period of time sufficient to form posterior foregut spheroids; and either c1) contacting the posterior foregut spheroids with retinoic acid, EGF, and a BMP signaling pathway inhibitor for a period of time sufficient to form the gastric tissue, wherein the gastric tissue is antrum gastric tissue; or c2) contacting the posterior foregut spheroids with retinoic acid, EGF, a BMP signaling pathway inhibitor and a WNT signaling pathway activator for a period of time sufficient to form the gastric tissue, wherein the gastric tissue is fundus gastric tissue.

[0006b] In one aspect provided herein is a gastric tissue produced by the method as described herein.

[0006c] In one aspect provided herein is an *in vitro* method for identifying the absorption effect of a gastric tissue, comprising the steps of: a) contacting the gastric tissue with a compound, wherein said gastric tissue is produced *in vitro* by the method as described herein; and b) detecting a level of absorption of said compound by said gastric 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 publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0009] **FIG 1** depicts expression of Sox2/Cdx2/B-catenin in gastric spheroids and the effect of RA.

[0010] **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).

[0011] **FIG 3A-3D** depictsMuc5AC, TFF2, GSII UEAI, and CHGA expression in P12 Antrum, E18.5 Antrum and d34 Organoid (FIG

Claims

What is claimed is:

1. ~~A~~ An *in vitro* method of inducing formation of a gastric tissue, comprising the steps of:

a) ~~activating one or more signaling pathways within a precursor cell, wherein said one or more signaling pathways are selected from~~contacting a definitive endoderm cell with a WNT signaling pathway~~, a WNT/~~ activator, an FGF signaling pathway~~, a FGF signaling pathway, or a combination thereof;~~

~~b) inhibiting a BMP signaling pathway within said precursor cell;~~ activator, and a BMP signaling pathway inhibitor for a period of time sufficient to form SOX2+ foregut cells;

~~c) administering retinoic acid to said precursor cell.~~

b) contacting the SOX2+ foregut cells with retinoic acid for a period of time sufficient to form posterior foregut spheroids; and either

c1) contacting the posterior foregut spheroids with retinoic acid, EGF, and a BMP signaling pathway inhibitor for a period of time sufficient to form the gastric tissue, wherein the gastric tissue is antrum gastric tissue; or

c2) contacting the posterior foregut spheroids with retinoic acid, EGF, a BMP signaling pathway inhibitor and a WNT signaling pathway activator for a period of time sufficient to form the gastric tissue, wherein the gastric tissue is fundus gastric tissue.

2. ~~–~~ The method of claim 1, wherein ~~said precursor cell is selected from an embryonic stem cell, an embryonic germ cell, an induced pluripotent stem cell, a mesoderm cell, a definitive endoderm cell, a posterior endoderm cell, a posterior endoderm cell, and~~ the gastric tissue is a ~~hindgut cell, preferably a~~gastric organoid.

3. The method of claim 2, wherein the definitive endoderm cell is 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.~~

4. The method of any one of claims 1 through ~~4~~3, wherein said ~~precursor cell is a~~ definitive endoderm cell is 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.~~

5. The method of any one of claims 1 through ~~3~~4, wherein said FGF ~~signalling~~signaling pathway activator 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, and FGF23~~, preferably FGF4, more preferably FGF4 at a concentration between about 50 to about 1500 ng/ml~~.

~~5.~~

6. The method of any one of claims 1 through ~~4~~5, wherein said FGF signaling pathway activator is FGF4.

7. The method of claim 6, wherein FGF4 is at a concentration between about 50 to about 1500 ng/mL.

8. The method of any one of claims 1 through 7, wherein said WNT ~~signalling~~signaling pathway activator is ~~activated using an agent~~ selected from one or more molecules selected from the group consisting of Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, Wnt8a, Wnt8b, Wnt9a, Wnt9b, Wnt10a, Wnt10b, Wnt11, ~~and Wnt16, preferably Wnt3a, more preferably Wnt3a at a concentration between about 50 to about 1500 ng/ml~~Wnt16, and a small molecule activator of the WNT signaling pathway.

~~6. The method of any of claims 1 through 5, wherein said activating step comprises contacting said precursor cell with both Wnt3a and FGF4 over a specified activation period.~~

~~7.~~

9. The method of claim 8, wherein said small molecule activator of the WNT signaling pathway is selected from the group consisting of: Lithium Chloride; 2-amino-4,6-disubstituted pyrimidine (hetero) arylpyrimidines; IQ1; QS11; DCA; beta-catenin; 2-amino-4-[3,4-

(methylenedioxy)-benzyl-amino]-6-(3-methoxyphenyl) pyrimidine; a GSK inhibitor; CHIR99021; WAY-316606; SB-216763 and BIO (6-bromoindirubin-3'-oxime).

10. The method of any one of claims 1 through 9, wherein said WNT signaling pathway activator is Wnt3a or CHIR99021.

11. The method of claim 10, wherein Wnt3 is at a concentration between about 50 and about 1500 ng/mL, or CHIR99021 is at a concentration of about 2 µM.

12. The method of any one of claims 1 through ~~6, wherein said inhibiting step comprises contacting said precursor cell with an agent that inhibits~~ 11, wherein the WNT signaling pathway activator is Wnt3a or CHIR99021 and the FGF pathway activator is FGF4.

13. The method of any one of claims 1 through 12, wherein the BMP signaling pathway~~, wherein said agent~~ inhibitor is selected from Noggin, Dorsomorphin, LDN189, DMH-1, and combinations thereof~~, preferably wherein said precursor cell is contacted with a BMP inhibitor at a concentration between about 50 to about 1500 ng/ml.~~ .

14.~~8.~~ The method of ~~any of claims 1 through 7~~claim 13, wherein ~~said steps a) through c) are carried out for a period of time sufficient to cause formation of a foregut spheroid, preferably~~the BMP signaling pathway inhibitor is Noggin.

15. The method of claim 14, wherein ~~steps a)~~Noggin is at a concentration between about 50 and ~~b) are carried out prior to step c).~~about 1500 ng/mL.

~~9.~~

16. The method of any one of claims 1 through 15, wherein the posterior foregut spheroids express PDX1, SOX2, HNF1beta, and HNF6.

17.~~8.~~ The method of any one of claims 1 through 16 wherein the retinoic acid ~~of step c)~~ is ~~administered~~contacted 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~~.

18.~~10.~~ The method of any one of claims 1 through ~~9~~17, wherein ~~steps~~step a) ~~and b) are~~is carried out for a period of about 1 to about 4 days, and wherein step ~~c~~b) is carried out for about ~~3~~1 to about ~~5~~2 days.

19.~~, preferably wherein steps a)~~ The method of any one of claims 1 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, preferably~~18, wherein ~~said exposure to EGF is at a concentration and length of time sufficient to increase said~~the gastric ~~spheroids to~~tissue is 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.

20.~~11.~~ The method of any one of claims 1 through ~~10, further comprising~~ 19, wherein the ~~step~~ antrum gastric tissue is characterized by expression of ~~culturing said activated precursor cell *in vitro* to form a 3-dimensional tissue structure~~MUC5AC, gastrin, ghrelin, 5-HT, and ChrA.

21.~~12.~~ The method of any one of claims 1 through ~~11, wherein said activating and inhibiting steps are conducted *in vitro*~~20, wherein the fundus gastric tissue is characterized by expression of IRX3 and IRX5, and repression of PDX1.

~~13. The~~

22. A gastric tissue produced by the method of any ~~of claims 1 through 12, wherein said activation period and said inhibition period overlap.~~one

~~14. The method of any~~ of claims 1~~ through 12, wherein said activation period and said inhibition period do not overlap~~ 21.

~~15.~~

23. The method of any one of claims 1-21, wherein step c1) is performed.

24. The method of any one of claims 1~~ through 14~~ 21, wherein ~~said activation period is between about 24 and about 120 hours~~step c2) is performed.

~~16. A composition comprising gastric tissue produced *in vitro* from one or more precursor cells.~~

~~17. The composition of claim 16, wherein the one or more precursor cells are selected from the group consisting of an embryonic stem cell, an embryonic germ cell, an induced pluripotent stem cell, a mesoderm cell, a definitive endoderm cell, a posterior endoderm cell, and a hindgut cell.~~

~~18. 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, an agent that activates the WNT pathway, an agent that inhibits the BMP pathway, 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, and an agent that inhibits the BMP pathway to generate a fundus tissue.~~

~~19. 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, an agent that activates the WNT pathway, an agent that inhibits the BMP pathway, 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 to generate an antrum tissue.~~

~~20. A kit comprising a gastric tissue produced *in vitro* from one or more precursor cells.~~

25.~~21. A~~ An *in vitro* method for identifying the absorption effect of a gastric tissue, comprising the steps of:

a) contacting the gastric ~~cells or tissues, comprising the steps of:~~

~~a) contacting gastric cells or tissues~~ tissue with a compound, wherein said gastric ~~cells or tissues are~~tissue is produced *in vitro* ~~from~~by the method of any one ~~or more precursor cells~~of claims 1-21; and

b) detecting a level of absorption of said compound by said gastric ~~cells or tissues~~tissue.