[0006] Furthermore, factors endowing liver cells the ability to engraft, proliferate and differentiate *in vivo* remain to be fully examined, wherein the difficulty in attaining adult-like liver cells is still widely experienced.

[0007] Altogether, although pluripotent stem cells could yield liver cells with some liver function, there remains an apparently un-surmounted barrier for these liver cells to progress into an adult-like state. Furthermore, generating 'authentic' and transplantable hepatocyte-like cells from these pluripotent stem cells that could engraft and robustly repopulate in the adult liver remains challenging.

[0008] In view of these challenges, it is important to understand the signaling logic underlying multiple steps of induction and patterning of the germ layers and differentiation into the various cell lineages. In particular, it is important to understand the signaling factors that regulate liver specification, and to formulate a signaling paradigm for hepatogenesis using human pluripotent stem cell-based differentiation.

[0009] It is an aim of the present invention to elucidate the underlying signaling logic of stem cell induction and differentiation in order to unilaterally drive stem cells to generate liver cells with minimal extraneous lineages.

SUMMARY

[0010] Disclosed is a method of differentiating cells of the definitive endoderm (DE) lineage into posterior foregut (PFG) lineage comprising contacting said stem cells with: one or more retinoic acid activators; and one or more inhibitors of TGFβ signaling.

[0011] Disclosed is a method of differentiating cells of the posterior foregut lineage into liver bud (LB) progenitors comprising contacting said cells of the posterior foregut lineage with one or more activators of TGFβ signaling, one or more modulators of Wnt signaling; and one or more activators of cyclic AMP/PKA signaling.

[0012] Disclosed is a method of differentiating liver bud progenitors into hepatic progenitors comprising contacting said liver bud progenitors with: one or more inhibitors of TGFβ signaling; one or more inhibitors of FGF signaling; and one or more inhibitors of Notch signaling.

[0012a] In one aspect, there is provided a method of differentiating liver bud progenitors into hepatic progenitors comprising: contacting said liver bud progenitors with one or more inhibitors of Notch signaling, optionally

further comprising contacting the cells with: one or more inhibitors of Notch signaling; one or more activators of ascorbic acid signaling, one or more activators of cyclic AMP/PKA signaling; and/or one or more activators of insulin signaling, optionally

wherein the duration of contact is for about 48 hours, optionally

wherein the duration of contact is for at least 1 to 108 hours, optionally further comprising contacting said liver bud progenitors with one or more differentiation factors selected from the group comprising of: activators of ascorbic acid signaling; activators of glucocorticoid signaling; activators of cyclic AMP/PKA signaling; activators of insulin signaling; activators of oncostatin M signaling; an amino acid mixture, and/or activators of L-glutathione signaling, optionally further comprising contacting the cells with one or more differentiation factors selected from the group comprising of: activators of BMP signaling; inhibitors of PKG signaling; activators of glucocorticoid signaling; phospholipid precursors; activators of oncostatin M signaling; an amino acid mixture, and activators of L-glutathione signaling, optionally

wherein the hepatic progenitors comprise elevated gene expression of hepatic markers and decreased expression of biliary markers relative to undifferentiated cells, optionally

wherein the hepatic progenitors comprise elevated gene expression of markers comprises ALBUMIN, c-MET, HNF4A, CEBPA or a combination thereof, relative to undifferentiated cells, optionally wherein the hepatic progenitors comprise decreased gene expression of biliary marker SOX9 relative to undifferentiated cells optionally,

wherein said liver bud progenitors are obtained from a method comprising:

contacting cells of the posterior foregut lineage with: one or more activators of TGFβ signaling, one or more modulators of Wnt signaling; and/or one or more activators of cyclic AMP/PKA signaling, optionally further comprising contacting said cells of the posterior foregut lineage with one or more activators of BMP signaling, optionally wherein the liver bud progenitors comprise elevated gene expression of liver bud

progenitor markers and decreased expression of pancreatic progenitor markers relative to undifferentiated cells, optionally wherein the liver bud progenitors comprise elevated gene expression of markers comprising AFP, TBX3, HNF4A PROX1, HNF1B, HNF6, CEBPAα or a combination thereof, relative to undifferentiated cells, optionally wherein the one or more modulators of Wnt signaling comprise an inhibitor of Wnt signaling and/or an activator of Wnt signaling, optionally

wherein the inhibitor of Wnt signaling is contacted with the cells of the posterior foregut lineage for duration of about 1 to 72 hours, and subsequently the activator of Wnt signaling is contacted with the cells of the posterior foregut lineage for duration of about 24 to 48 hours, optionally wherein the duration of the method is for about 1 to 120 hours, optionally wherein said cells of the posterior foregut lineage are obtained from a method comprising:

contacting stem cells of the definitive endoderm (DE) lineage with: one or more retinoic acid activators; and/or one or more inhibitors of TGFβ signaling, optionally further comprising contacting said stem cells with one or more activators of BMP signaling, optionally further comprising contacting said stem cells with one or more activators of FGF signaling, optionally wherein the cells of the posterior foregut lineage comprise elevated gene expression of posterior foregut lineage markers and decreased expression of dorsal foregut markers relative to undifferentiated cells, optionally wherein the duration of the method is about 1 to 84 hours.

[0013] Disclosed is a method of differentiating hepatic progenitors into perivenous hepatocytes comprising contacting said hepatic progenitors with:

one or more inhibitors of Notch signaling, one or more activators of glucocorticoid signaling, one or more activators of insulin signaling; one or more activators of ascorbic acid signaling, and one or more activators of TGFβ signaling.

[0014] Disclosed is a method of differentiating hepatic progenitors into hepatocyte-like cells comprising contacting said hepatic progenitors with: one or more activators of cyclic AMP/PKA signaling, one or more activators of glucocorticoid signaling; one or more activators of insulin signaling; one or more activators of ascorbic acid signaling, and one or more inhibitors of Notch signaling.

[0015] Disclosed is a method of maintaining liver bud progenitors in a self-renewal state comprising contacting said liver bud progenitors with: one or more activators of FGF signaling, and one or more activators of Wnt signaling.

[0016] Disclosed is a method of maintaining hepatocytes or hepatocyte-like cells in a perivenous state comprising contacting said cells with one or more inhibitors of Notch signaling and/or one or more activators of Wnt signaling.

[0017] Disclosed is a method of maintaining heaptocytes in a periportal state comprising contacting said cells with one or more activators of cyclic AMP/PKA signaling.

[0018] Disclosed is a kit for differentiating cells of the definitive endoderm (DE) lineage into posterior foregut lineage comprising one or more retinoic acid activators, and one or more inhibitors of TGFβ signaling.

[0019] Disclosed is a kit for differentiating cells of the posterior foregut lineage into liver bud progenitors comprising one or more activators of TGFβ signaling; one or more modulators of Wnt signaling; and/or one or more activators of cyclic AMP/PKA signaling.

[0020] Disclosed is a kit for differentiating liver bud progenitors into hepatic progenitors comprising one or more inhibitors of TGFβ signaling; one or more inhibitors of FGF signaling; and/or one or more inhibitors of Notch signaling.

[0021] Disclosed is a kit for differentiating liver bud progenitors into hepatic progenitors comprising one or more inhibitors of Notch signaling; activators of ascorbic acid signaling; one or more activators of cyclic AMP/PKA signaling; and/or one or more activators of insulin signaling.

[0022] Disclosed is a kit for differentiating hepatic progenitors into perivenous hepatocyte-like cells comprising one or more inhibitors of Notch signaling; one or more activators of glucocorticoid signaling; one or more activators of insulin signaling; one or more activators of ascorbic acid signaling; and/or one or more activators of TGFβ signaling.

[0023] Disclosed is a kit for differentiating hepatic progenitors into hepatocytes or hepatocyte-like cells comprising one or more activators of cyclic AMP/PKA signaling; one or more activators of glucocorticoid signaling; one or more activators of insulin signaling; one or more activators of ascorbic acid signaling; and/or one or more inhibitors of Notch signaling.

[0024] Disclosed is a kit for maintaining liver bud progenitors in a self-renewal state comprising one or more of the following factors: one or more activators of FGF signaling and/or one or more activators of Wnt signaling.

[0025] Disclosed is a kit for maintaining hepatocytes or hepatocyte-like cells in a perivenous state comprising one or more of the following factors: one or more inhibitors of Notch signaling and/or one or more activators of Wnt signaling.

[0026] Disclosed is a kit for maintaining hepatocytes or hepatocyte-like cells in a periportal state comprising one or more activators of cyclic AMP/PKA signaling.

[0027] Disclosed is a surface marker for isolating or selecting for LB cells selected from EGFR or CD99.

[0028] Disclosed is a surface marker for isolating or selecting for MHG cells comprising CD325 (N-cadherin).

[0029] Disclosed is a method of screening for a cellular response, the method comprising: a) contacting a population of cells generated according to the methods as described herein with a pharmacological agent; and b) evaluating the population of cells for a cellular response induced by the pharmacological agent.

[0030] Disclosed is a method of screening for a phenotype, the method comprising: a) administering to a host animal a population of cells generated according to the methods as described herein, wherein the cells of the population of cells comprise a genetic modification in at least one genetic locus; and b) evaluating the host animal for a detectable phenotype induced by the administered population of cells.

[0031] Disclosed is a method of treating a subject for a condition, the method comprising: a) administering the subject a therapeutically effective amount of cells generated according to the methods described herein in order to treat the subject for the condition.

[0032] Disclosed is use of a therapeutically effective amount of cells generated according to the methods as described herein in the manufacture of a medicament for treating a condition in a subject. In another aspect, there is provided use of a therapeutically effective amount of cells generated according to the methods described herein in the manufacture of a medicament for treating a subject for loss of liver function, wherein the medicament is to be administered to the subject, optionally wherein the medicament is to be co-administered with at least one pro-survival or pro-engraftment factor, optionally wherein the cells comprise a genetic modification in at least one genetic locus.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

Definitions

[0033] The following words and terms used herein shall have the meaning indicated:

[0034] As used herein, the term "stem cells" include but are not limited to undifferentiated cells defined by their ability at the single cell level to both self-renew and differentiate to produce progeny cells, including self-renewing progenitors, non-renewing progenitors, and terminally differentiated cells. For example, "stem cells" may include (1) totipotent stem cells; (2) pluripotent stem cells; (3) multipotent stem cells; (4) oligopotent stem cells; and (5) unipotent stem cells.

[0035] As used herein, the term "pluripotent stem cell" (PSC) refers to a cell with the developmental potential, under different conditions, to differentiate to cell types characteristic of all three germ cell layers, i.e., endoderm (e.g., gut tissue), mesoderm (including blood, muscle, and vessels), and ectoderm (such as skin and nerve). The developmental competency of a cell to differentiate to all three germ layers can be determined using, for example, a nude mouse teratoma formation assay. In some embodiments, pluripotency can also be evidenced by the expression of embryonic stem (ES) cell markers, although the preferred test for pluripotency of a cell or population of cells generated using the compositions and methods described herein is the demonstration that a cell has the developmental potential to differentiate into cells of each of the three germ layers.

[0036] As used herein, the term "induced pluripotent stem cells" or, iPSCs, means that the stem cells are produced from differentiated adult cells that have been induced or changed, i.e., reprogrammed into cells capable of differentiating into tissues of all three germ or dermal layers: mesoderm, endoderm, and ectoderm. The iPSCs produced do not refer to cells as they are found in nature.

WHAT IS CLAIMED:

1. A method of differentiating liver bud progenitors into hepatic progenitors comprising: contacting said liver bud progenitors with one or more inhibitors of Notch signaling, optionally

further comprising contacting the cells with: one or more inhibitors of Notch signaling; one or more activators of ascorbic acid signaling, one or more activators of cyclic AMP/PKA signaling; and/or one or more activators of insulin signaling, optionally

wherein the duration of contact is for about 48 hours, optionally

wherein the duration of contact is for at least 1 to 108 hours, optionally further comprising contacting said liver bud progenitors with one or more differentiation factors selected from the group comprising of: activators of ascorbic acid signaling; activators of glucocorticoid signaling; activators of cyclic AMP/PKA signaling; activators of insulin signaling; activators of oncostatin M signaling; an amino acid mixture, and/or activators of L-glutathione signaling, optionally further comprising contacting the cells with one or more differentiation factors selected from the group comprising of: activators of BMP signaling; inhibitors of PKG signaling; activators of glucocorticoid signaling; phospholipid precursors; activators of oncostatin M signaling; an amino acid mixture, and activators of L-glutathione signaling, optionally wherein the hepatic progenitors comprise elevated gene expression of hepatic markers and decreased expression of biliary markers relative to undifferentiated cells, optionally

wherein the hepatic progenitors comprise elevated gene expression of markers comprises ALBUMIN, c-MET, HNF4A, CEBPA or a combination thereof, relative to undifferentiated cells, optionally wherein the hepatic progenitors comprise decreased gene expression of biliary marker SOX9 relative to undifferentiated cells optionally,

wherein said liver bud progenitors are obtained from a method comprising:

contacting cells of the posterior foregut lineage with: one or more activators of TGFβ signaling, one or more modulators of Wnt signaling; and/or one or more activators of cyclic AMP/PKA signaling, optionally further comprising contacting said cells of the posterior foregut lineage with one or more activators of BMP signaling, optionally wherein the liver bud progenitors comprise elevated gene expression of liver bud

progenitor markers and decreased expression of pancreatic progenitor markers relative to undifferentiated cells, optionally wherein the liver bud progenitors comprise elevated gene expression of markers comprising AFP, TBX3, HNF4A PROX1, HNF1B, HNF6, CEBPAα or a combination thereof, relative to undifferentiated cells, optionally wherein the one or more modulators of Wnt signaling comprise an inhibitor of Wnt signaling and/or an activator of Wnt signaling, optionally

wherein the inhibitor of Wnt signaling is contacted with the cells of the posterior foregut lineage for duration of about 1 to 72 hours, and subsequently the activator of Wnt signaling is contacted with the cells of the posterior foregut lineage for duration of about 24 to 48 hours, optionally wherein the duration of the method is for about 1 to 120 hours, optionally wherein said cells of the posterior foregut lineage are obtained from a method comprising:

contacting stem cells of the definitive endoderm (DE) lineage with: one or more retinoic acid activators; and/or one or more inhibitors of TGFβ signaling, optionally further comprising contacting said stem cells with one or more activators of BMP signaling, optionally further comprising contacting said stem cells with one or more activators of FGF signaling, optionally wherein the cells of the posterior foregut lineage comprise elevated gene expression of posterior foregut lineage markers and decreased expression of dorsal foregut markers relative to undifferentiated cells, optionally wherein the duration of the method is about 1 to 84 hours.

2. The method of claim 1, wherein the hepatic progenitors are further differentiated into perivenous hepatocyte-like cells comprising contacting said hepatic progenitors with: one or more inhibitors of Notch signaling, one or more activators of glucocorticoid signaling, one or more activators of insulin signaling; one or more activators of ascorbic acid signaling, and/or one or more activators of TGFβ signaling, optionally further comprising contacting the hepatic progenitors with one or more activators of retinoic acid signaling, optionally further comprising contacting said hepatic progenitors with one or more activators of progesterone signaling, optionally further comprising contacting said hepatic progenitors with one or more activators of vitamin D signaling, optionally further comprising contacting said hepatic progenitors with one or more activators of PKG signaling, optionally wherein the

perivenous hepatocyte-like cells comprise elevated gene expression of perivenous hepatocytes markers and decreased expression of hepatocyte-like cell markers relative to undifferentiated cells, optionally wherein the perivenous hepatocyte markers comprise GS, CYP3A4, AAT, AXIN2 or a combination thereof, optionally wherein the duration of the method is about 1 to 168 hours, optionally wherein said hepatic progenitors are obtained from the method of claim 1.

3. The method of claim 2, wherein the hepatic progenitors are further differentiated into hepatocyte-like cells comprising contacting said hepatic progenitors with: one or more activators of cyclic AMP/PKA signaling, one or more activators of glucocorticoid signaling; one or more activators of insulin signaling; one or more activators of ascorbic acid signaling, and/or one or more inhibitors of Notch signaling, optionally further comprising contacting said hepatic progenitors with one or more modulators of Wnt signaling, optionally wherein the hepatocyte-like cells comprise elevated gene expression of hepatocyte-like cell markers and decreased expression of perivenous markers relative to undifferentiated cells, optionally wherein the hepatocyte-like cell markers comprise FAH, PAH, HGD, HPD, TAT, Albumin, AAT, ARG1, CPS1 or a combination thereof, optionally wherein the duration of the method is for about 1 to 168 hours, optionally wherein said hepatic progenitors are obtained from the method of claim 1.

4. The method of any one of claims 1 to 3, wherein the liver bud progenitors are maintained in a self-renewal state by contacting said liver bud progenitors with: one or more activators of FGF signaling; and/or one or more activators of Wnt signaling, optionally further comprising contacting said liver bud progenitors with one or more activators of BMP signaling optionally,
further comprising contacting said liver bud progenitors with one or more epidermal growth factors, optionally further comprising contacting said liver bud progenitors with one or more modulators of Notch signaling, optionally wherein said liver bud progenitors are obtained from a method comprising:
contacting cells of the posterior foregut lineage with: one or more activators of TGFβ signaling, one or more modulators of Wnt signaling; and/or one or more activators of cyclic AMP/PKA signaling, optionally further comprising contacting said cells of the posterior foregut lineage with one or more activators of BMP signaling, optionally wherein the liver bud progenitors comprise elevated gene expression of liver bud

progenitor markers and decreased expression of pancreatic progenitor markers relative to undifferentiated cells, optionally wherein the liver bud progenitors comprise elevated gene expression of markers comprising AFP, TBX3, HNF4A PROX1, HNF1B, HNF6, CEBPAα or a combination thereof, relative to undifferentiated cells, optionally wherein the one or more modulators of Wnt signaling comprise an inhibitor of Wnt signaling and/or an activator of Wnt signaling, optionally

wherein the inhibitor of Wnt signaling is contacted with the cells of the posterior foregut lineage for duration of about 1 to 72 hours, and subsequently the activator of Wnt signaling is contacted with the cells of the posterior foregut lineage for duration of about 24 to 48 hours, optionally wherein the duration of the method is for about 1 to 120 hours, optionally wherein said cells of the posterior foregut lineage are obtained from a method comprising:

contacting stem cells of the definitive endoderm (DE) lineage with: one or more retinoic acid activators; and/or one or more inhibitors of TGFβ signaling, optionally further comprising contacting said stem cells with one or more activators of BMP signaling, optionally further comprising contacting said stem cells with one or more activators of FGF signaling, optionally wherein the cells of the posterior foregut lineage comprise elevated gene expression of posterior foregut lineage markers and decreased expression of dorsal foregut markers relative to undifferentiated cells, optionally wherein the duration of the method is about 1 to 84 hours.

5. Use of a therapeutically effective amount of cells generated according to any one of Claims 1-4 in the manufacture of a medicament for treating a subject for loss of liver function, wherein the medicament is to be administered to the subject, optionally wherein the medicament is to be co-administered with at least one pro-survival or pro-engraftment factor, optionally wherein the cells comprise a genetic modification in at least one genetic locus.