INDUCED HEPATOCYTES AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/085,185, filed on November 26, 2014, which is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE

[0002] All publications, patents, and patent applications disclosed herein are incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. In the event of a conflict between a term disclosed herein and a term in an incorporated reference, the term herein controls.

SUMMARY

[0003] In one of many examples, disclosed herein is a method of inducing a trophoblast stem (TS) cell to differentiate into an induced hepatocyte *in vitro*, comprising: contacting the trophoblast stem cell with a conditioned medium (e.g., for sufficient time) to induce differentiation of the trophoblast stem cell into an induced hepatocyte, wherein the condition medium comprises a fibroblast growth factor (FGF), a steroid, and a cytokine. In some examples, disclosed herein is a method of inducing a trophoblast stem (TS) cell to differentiate into an induced hepatocyte *in vitro*, which comprises (a) contacting the trophoblast stem cell in a conditioned medium comprising a fibroblast growth factor (FGF), a steroid, and a cytokine; and (b) incubating the cell for sufficient time to induce differentiation of the trophoblast stem cell into an induced hepatocyte. In some examples, the method further comprises contacting the trophoblast stem cell with the FGF prior to addition of the steroid and the cytokine to the conditioned medium. In some examples, the trophoblast stem cell is contacted with FGF for at least 2 hours, at least 4 hours, at least 6 hours, 8 hours, at least 12 hours, at least 16 hours, at least 20 hours, or at least 24 hours prior to addition of the steroid and the cytokine to the conditioned medium. In some examples, the method further comprises incubating the trophoblast stem cell for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, or at least 7 days. In some examples, the steroid and the cytokine are added simultaneously or sequentially into the conditioned medium.

[0003a] In another example, disclosed herein is an isolated induced hepatocyte derived from a trophoblast stem cell, wherein the hepatocyte expresses one or more biomarkers selected from

the group consisting of human leukocyte antigen G (HLA-G), transforming growth factor beta 1 (TGFβ1), cytochrome P450 family 1 subfamily A polypeptide 1 (CYP1A1), C-X-C chemokine receptor type 4 (CXCR4), bile salt export pump (BSEP), forkhead box protein A2 (FOXA2), hematopoietically expressed homeobox (HHEX), cytochrome P450 7A1 (CYP7A1), serpin peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin) member 1 (SERPINA1), ATP-binding cassette sub-family C (ABCC2), CCAAT-enhancer-binding protein beta (C/EBPβ), and hepatocyte nuclear factor 1-alpha (HNF1α).

[0003b] In yet another example, disclosed herein is a composition comprising the isolated hepatocyte disclosed herein.

[0003c] In yet another example, disclosed herein is a pharmaceutical composition comprising the isolated hepatocyte disclosed herein.

[0003d] In yet another example, disclosed herein is use of the isolated hepatocyte disclosed herein in the manufacture of a medicament for the treatment of a liver-associated disease or disorder in a subject.

[0003e] In yet another example, disclosed herein is a composition comprising the hepatocyte of claim 1 in the manufacture of a medicament for liver regeneration.

[0004] In some examples, an induced hepatocyte herein is a hepatic progenitor cell. In some examples, FGF upregulates miRNA-124a in the TS cell. In some examples, elevated level of miRNA-124a initiates definitive endoderm (DE) specification in the TS cell. In some

embodiments, the DE specification is associated with biomarkers comprising forkhead box protein A2 (FOXA2), SRY-box 17 (SOX17), Goosecoid (GSC), or Homeodomain protein MIXL1. In some examples, the DE specification is associated with elevated expression levels of SOX17, FOXA2, and GSC. In some examples, the elevated expression levels are increased protein expression levels. In some examples, the DE specification is associated with a decreased expression level of MIXL1. In some examples, the decreased expression level is a decreased protein expression levels. In some examples, the elevated protein expression levels of SOX17, FOXA2, and GSC and the decreased protein expression level of MIXL1 are relative to the protein expression levels of SOX17, FOXA2, GSC, and MIXL1 in an equivalent TS cell that has not undergone DE specification. In some examples, the DE specification is further associated with elevated expression levels of SOX2, NANOG, and OCT4. In some examples, elevated expression levels of SOX2, NANOG, and OCT4 are increased level of protein expressions. In some examples, elevated expression levels of SOX2, NANOG, and OCT4 are increased level of gene expressions. In some examples, the elevated expression levels of SOX2, NANOG, and OCT4 are relative to the expression levels of SOX2, NANOG, and OCT4 in an equivalent TS cell that has not undergone DE specification. In some examples, differentiation induced by a method herein comprises one or more of four stages: primitive streak to definitive endoderm (DE) stage, hepatic specified endoderm stage, hepatoblastic stage, and the fetal and adult hepatocyte cell stage. In some examples, one or more biomarkers selected from the group consisting of CXCR4, FOXA2, SOX17, HHEX, TTR, ALB, TAT, CYP7A1, BSEP, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, HNF4α, and any combination thereof express in one or more of the four stages. In some examples, one or more biomarkers selected from the group consisting of CXCR4, FOXA2, SOX17, HHEX, and any combination thereof, express at the primitive streak to DE stage. In some examples, an expression level of CXCR4, FOXA2, SOX17, and/or HHEX increases at the primitive streak to DE stage, relative to that before the primitive streak to DE stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of CXCR4, FOXA2, SOX17 and/or HHEX increases by about 1 fold and about 10,000 fold higher than that before the primitive streak to DE stage. In some examples, the expression level of CXCR4, FOXA2, SOX17 and/or HHEX increases by about 10 fold and about 1000 fold higher than that before the primitive streak to DE stage. In some examples, one or more biomarkers selected from the group consisting of SOX17, TTR, ALB, TAT, SERPINA1, CYP7A1, and any combination thereof express in the hepatic specified endoderm stage. In some examples, an expression level of SOX17, TTR, ALB, TAT, SERPINA1,

and/or CYP7A1 increases at the hepatic specified endoderm stage, relative to that before the hepatic specified endoderm stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of SOX17, TTR, ALB, TAT, SERPINA1, and/or CYP7A1 increases by about 1 fold and about 1000 fold higher than that before the hepatic specified endoderm stage. In some examples, the expression level of SOX17, TTR, ALB, TAT, SERPINA1, and/or CYP7A1 increases by about 10 fold and about 100 fold higher than that before the hepatic specified endoderm stage. In some examples, one or more biomarkers selected from the group consisting of TTR, ALB, TAT, CYP7A1, SERPINA1, BSEP, and any combination thereof express at the hepatoblastic stage. In some examples, an expression level of TTR, ALB, TAT, CYP7A1, SERPINA1, and/or BSEP increases at the hepatoblastic stage, relative to that before the hepatoblastic stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of TTR, ALB, TAT, CYP7A1, SERPINA1, and/or BSEP increases by about 1 fold and about 1000 fold higher than that before the hepatoblastic stage. In some examples, the expression level of TTR, ALB, TAT, CYP7A1, SERPINA1, and/or BSEP increases by about 10 fold and about 100 fold higher than that before the hepatoblastic stage. In some examples, one or more biomarkers selected from the group consisting of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, HNF4α, and any combination thereof express at the fetal and adult hepatocyte-like cell stage. In some examples, an expression level of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, and/or HNF4α increases at the fetal and adult hepatocyte-like cell stage, relative to that before the fetal and adult hepatocyte-like cell stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, and/or HNF4α increases by about 10 fold and about 1000 fold higher than that before the fetal and adult hepatocyte cell stage. In some examples, the expression level of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, and/or HNF4α increases by at least 100 fold higher than that before the fetal and adult hepatocyte cell stage.

[0005] In some examples, a trophoblast stem cell herein is a human trophoblast stem cell. In some examples, the steroid is dexamethasone. In some examples, the cytokine is oncostatin M. In some examples, the oncostatin M is a human oncostatin M. In some examples, the human oncostatin M is a recombinant human oncostatin M. In some examples, the conditioned medium further comprises a bone morphogenetic protein (BMP). In some examples, the BMP is present in a concentration of about 1-100 ng/ml. In some

examples, the BMP is present in a concentration of about 1-50 ng/ml, e.g., about 20 ng/ml. In some examples, the BMP is BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP10, or BMP15. In some examples, the BMP is BMP4. In some examples, the conditioned medium further comprises a hepatic growth factor (HGF). In some examples, the HGF is present in a concentration of about: 0.1-50 ng/ml or 0.1-25 ng/ml, e.g., about 5 ng/ml.

[0006] In some examples, a hepatocyte disclosed herein is immune privileged. In some examples, the induced hepatocyte expresses TGFβ1. In some examples, the induced hepatocyte expresses TGFβ1, fibronectin, and collagen IV in extracellular matrix (ECM). In some examples, the induced hepatocyte expresses HLA-G. In some examples, the induced hepatocyte expresses HLA-G and stem-121. In some examples, the induced hepatocytes recruit $CD4^+Foxp3^+$ Treg cells. In some examples, the induced hepatocytes form tissue of a 3-dimensional structure. In some examples, the induced hepatocytes cluster or aggregate. In some examples, the induced hepatocytes form a crescent cell mass. In some examples, the induced hepatocytes comprise a peripheral compartment and a central compartment. In some examples, the induced hepatocytes distribute irregularly along ECM beyond basement membrane in the peripheral compartment. In some examples, the induced hepatocytes distribute from basal towards central areas in the central compartment. In some examples, the induced hepatocyte expresses one or more markers selected from the group consisting of TGFβ1, HLA-G, stem 121, C-kit, CK19, CK18, ALB, α-AFP, betatrophin, ADH1, APOF, CPS1, GATA4, CYP1A1, CYP2B6, ASGR1, CXCR4, BSEP, MRP2, Cx32, and any combination thereof. In some examples, the induced hepatocyte expresses one or more markers selected from the group consisting of TGFβ1, HLA-G, stem 121, C-kit, betatrophin, ADH1, APOF, CPS1, CYP2B6, ASGR1, CXCR4, Cx32, and any combination thereof. In some examples, the induced hepatocyte expresses one or more markers selected from the group consisting of CPS1, CYP2B6, and a combination thereof.

[0007] In one example, provided herein is an induced hepatocyte produced by any method disclosed herein. In another example, provided herein is an isolated induced hepatocyte derived from a trophoblast stem cell. In another example, provided herein is an isolated hepatocyte induced from a trophoblast stem cell. In some examples, the hepatocyte expresses one or more biomarkers selected from the group consisting of transforming growth factor beta 1 (TGFβ1), human leukocyte antigen G (HLA-G), cluster of differentiation 4 (CD4), forkhead box P3 (Foxp3), human cytoplasmic marker stem 121 (stem 121), mast/stem cell growth factor receptor C-kit (C-kit), betatrophin, apolipoprotein F (APOF), alcohol dehydrogenase-1 (ADH1),

carbamoyl-phosphate synthase 1 (CPS1), GATA transcription factor 4 (GATA4), cytochrome P450 family 1 subfamily A polypeptide 1 (CYP1A1), cytochrome P450 2B6 (CYP2B6), asialoglycoprotein receptor 1 (ASGR1), C-X-C chemokine receptor type 4 (CXCR4), bile salt export pump (BSEP), multi-drug resistance protein-2 (MRP2), connexin 32 (CX32), forkhead box protein A2 (FOXA2), SRY-box 17 (SOX17), hexosaminidase A alpha polypeptide (HEXA), hematopoietically expressed homeobox (HHEX), transthyretin (TTR), albumin (ALB), tyrosine aminotransferase (TAT), cytochrome P450 7A1 (CYP7A1), glucose-6-phosphatase (G6PC), serpin peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin) member 1 (SERPINA1), ATP-binding cassette sub-family C (ABCC2), CCAAT-enhancer-binding protein beta (C/EBPβ), hepatocyte nuclear factor 1-alpha (HNF1α), hepatocyte nuclear factor 4-alpha (HNF4α), alpha-1-fetoprotein (AFP), cytokeratin 8 (CK8), phosphoenolpyruvate carboxykinase 2 mitochondrial (PCK2), glycogen synthase 2 (GYS2), hepatocyte nuclear factor 6 (HNF6), alcohol dehydrogenase 1C (class I) gamma polypeptide (ADH1C), cytochrome P450 3A4 (CYP3A4), prospero homeobox 1 (PROX1), tryptophan 2,3-dioxygenase (TDO2), cytokeratin 18 (CK18), and cytokeratin 19 (CK19).

[0008] In some examples, a hepatocyte herein is a hepatic progenitor cell. In some examples, FGF upregulates miRNA-124a in the TS cell. In some examples, elevated level of miRNA-124a initiates definitive endoderm (DE) specification in the TS cell. In some examples, the DE specification is associated with biomarkers comprising forkhead box protein A2 (FOXA2), SRY-box 17 (SOX17), Goosecoid (GSC), or Homeodomain protein MIXL1. In some examples, the DE specification is associated with elevated expression levels of SOX17, FOXA2, and GSC. In some examples, the elevated expression levels are increased protein expression levels. In some examples, the DE specification is associated with a decreased expression level of MIXL1. In some examples, the decreased expression level is a decreased protein expression levels. In some examples, the elevated protein expression levels of SOX17, FOXA2, and GSC and the decreased protein expression level of MIXL1 are relative to the protein expression levels of SOX17, FOXA2, GSC, and MIXL1 in an equivalent TS cell that has not undergone DE specification. In some examples, the DE specification is further associated with elevated expression levels of SOX2, NANOG, and OCT4. In some examples, elevated expression levels of SOX2, NANOG, and OCT4 are increased level of protein expressions. In some examples, elevated expression levels of SOX2, NANOG, and OCT4 are increased level of gene expressions. In some examples, the elevated expression levels of SOX2, NANOG, and OCT4 are relative to the expression levels of SOX2, NANOG, and OCT4 in an equivalent TS cell that has not undergone DE specification. In

some examples, a hepatocyte disclosed herein is at one of four stages: primitive streak to definitive endoderm (DE) stage, hepatic specified endoderm stage, hepatoblastic stage, and the fetal and adult hepatocyte cell stage. In some examples, one or more biomarkers selected from the group consisting of CXCR4, FOXA2, SOX17, HHEX, TTR, ALB, TAT, CYP7A1, BSEP, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, HNF4α, and any combination thereof express in one or more of the four stages. In some examples, one or more biomarkers selected from the group consisting of CXCR4, FOXA2, SOX17, HHEX, and any combination thereof, express at the primitive streak to DE stage. In some examples, an expression level of CXCR4, FOXA2, SOX17, and/or HHEX increases at the primitive streak to DE stage, relative to that before the primitive streak to DE stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of CXCR4, FOXA2, SOX17 and/or HHEX increases by about 1 fold and about 10,000 fold higher than that before the primitive streak to DE stage. In some examples, the expression level of CXCR4, FOXA2, SOX17 and/or HHEX increases by about 10 fold and about 1000 fold higher than that before the primitive streak to DE stage. In some examples, one or more biomarkers selected from the group consisting of SOX17, TTR, ALB, TAT, SERPINA1, CYP7A1, and any combination thereof express in the hepatic specified endoderm stage. In some examples, an expression level of SOX17, TTR, ALB, TAT, SERPINA1, and/or CYP7A1 increases at the hepatic specified endoderm stage, relative to that before the hepatic specified endoderm stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of SOX17, TTR, ALB, TAT, SERPINA1, and/or CYP7A1 increases by about 1 fold and about 1000 fold higher than that before the hepatic specified endoderm stage. In some examples, the expression level of SOX17, TTR, ALB, TAT, SERPINA1, and/or CYP7A1 increases by about 10 fold and about 100 fold higher than that before the hepatic specified endoderm stage. In some examples, one or more biomarkers selected from the group consisting of TTR, ALB, TAT, CYP7A1, SERPINA1, bile salts excretion pump (BSEP), and any combination thereof express at the hepatoblastic stage. In some examples, an expression level of TTR, ALB, TAT, CYP7A1, SERPINA1, and/or BSEP increases at the hepatoblastic stage, relative to that before the hepatoblastic stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of TTR, ALB, TAT, CYP7A1, SERPINA1, and/or BSEP increases by about 1 fold and about 1000 fold higher than that before the hepatoblastic stage. In some examples, the expression level of TTR, ALB, TAT, CYP7A1, SERPINA1, and/or BSEP increases by about 10 fold and about 100 fold higher than that before the hepatoblastic

stage. In some examples, one or more biomarkers selected from the group consisting of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, HNF4α, and any combination thereof express at the fetal and adult hepatocyte-like cell stage. In some examples, an expression level of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, and/or HNF4α increases at the fetal and adult hepatocyte-like cell stage, relative to that before the fetal and adult hepatocyte-like cell stage. In some examples, the increased expression level is an increased level of gene expression. In some examples, the expression level of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, and/or HNF4α increases by about 10 fold and about 1000 fold higher than that before the fetal and adult hepatocyte cell stage. In some examples, the expression level of HHEX, BSEP, TTR, ALB, TAT, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, and/or HNF4α increases by at least 100 fold higher than that before the fetal and adult hepatocyte cell stage. In some examples, the trophoblast stem cell is a human trophoblast stem cell.

[0009] In some examples, a hepatocyte herein is immune privileged. In some examples, the hepatocyte expresses TGFβ1. In some examples, the hepatocyte expresses TGFβ1, fibronectin, and collagen IV in extracellular matrix (ECM). In some examples, the hepatocyte expresses HLA-G. In some examples, the hepatocyte expresses HLA-G and stem-121. In some examples, the hepatocyte recruits $CD4^+Foxp3^+$ Treg cells. In some examples, the hepatocytes form tissue of a 3-dimensional structure. In some examples, the hepatocytes cluster or aggregate. In some examples, the hepatocytes form a crescent cell mass. In some examples, the hepatocytes comprise a peripheral compartment and a central compartment. In some examples, the hepatocytes distribute irregularly along ECM beyond basement membrane in the peripheral compartment. In some examples, the hepatocytes distribute from basal towards central areas in the central compartment. In some examples, the hepatocyte expresses one or more markers selected from the group consisting of TGFβ1, HLA-G, stem 121, C-kit, CK19, CK18, ALB, α-AFP, betatrophin, ADH1, APOF, CPS1, GATA4, CYP1A1, CYP2B6, ASGR1, CXCR4, BSEP, MRP2, Cx32, and any combination thereof. In some examples, the hepatocyte expresses one or more markers selected from the group consisting of TGFβ1, HLA-G, stem 121, C-kit, betatrophin, ADH1, APOF, CPS1, CYP2B6, ASGR1, CXCR4, Cx32, and any combination thereof. In some examples, the hepatocyte expresses one or more markers selected from the group consisting of CPS1, CYP2B6, and a combination thereof. In some examples, the hepatocyte expresses one or more markers selected from the group consisting of stem 121, C-kit, CK19, CK18, and any combination thereof. In some examples, the hepatocyte expresses one or more markers selected from the

group consisting of ALB, AFP, betatrophin, ADH1, APOF, CPS1, GATA4, CYP1A1, CYP2B6, and any combination thereof. In some examples, the hepatocyte expresses one or more markers selected from the group consisting of ASGR1, CXCR4, BSEP, MRP2, Cx32, and any combination thereof.

[0010] Also disclosed herein is a method of screening a therapeutic compound for use in treatment or prevention of a condition, comprising: contacting an isolated hepatocyte disclosed herein with the therapeutic compound; and detecting an expression level of a biomarker in the isolated hepatocyte. In some examples, the expression level of a biomarker in the isolated hepatocyte increases as compared to an equivalent isolated hepatocyte not contacted with the therapeutic compound. In some examples, the expression level of a biomarker in the isolated hepatocyte decreases as compared to an equivalent isolated hepatocyte not contacted with the therapeutic compound. In some examples, the expression level is a gene expression level. In some examples, the biomarker comprises CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP3A4, or CYP7A1. In some examples, the therapeutic compound is a small molecule drug, a peptide, or a protein. In some examples, the therapeutic compound is a synthetic chemical drug. In some examples, the condition is a liver failure. In some examples, the condition is a liver-associated disease or disorder. In some examples, the liver-associated disease or disorder comprises alagille syndrome, alpha 1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, biliary atresia, cirrhosis, cystic disease of the liver, fatty liver disease including alcohol-related liver disease and non-alcohol fatty liver disease (NAFLD), galactosemia, gallstones, Gilbert's Syndrome, hemochromatosis, liver cysts, liver cancer, liver disease in pregnancy (optionally acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, preeclampsia, or HELLP Syndrome (hemolysis, elevated liver tests, low platelets)), neonatal hepatitis, primary billary cirrhosis, primary sclerosing cholangitis, porphyria, Reye's Syndrome, sarcoidosis, toxic hepatitis, type 1 glocogen storage disease, tyrosinemia, viral hepatitis, Wilson disease, or any combination thereof.

[0011] In one example, disclosed herein is a composition (e.g., pharmaceutical composition) comprising any hepatocyte disclosed herein.

[0012] In another example, disclosed herein is a method of treating a condition in a subject, comprising administering to a subject a pharmaceutical composition that comprises an isolated hepatocyte herein, in an amount effective for the hepatocytes to engraft to the subject (e.g., to the subject's liver). In some examples, the hepatocytes are administered in a pharmaceutically acceptable carrier. In some examples, the pharmaceutically acceptable carrier comprises a phosphate buffer saline. In some examples, the hepatocytes are administered in a suspension

containing about $1x10^6$ to about $100x10^6$ cells per ml, about $1x10^6$ to about $250x10^6$ cells per ml, about $1x10^6$ to about $500x10^6$ cells per ml, or about $10x10^6$ to about $40x10^6$ cells per ml. In some examples, the hepatocytes are administered in a volume of about: 1-5 ml, 1-10 ml, 1-50 ml, 1-100 ml, or 10-150 ml. In some examples, the subject is a human. In some examples, the administering comprises an injection, e.g., intravenous injection. In some examples, the injection is administered at a hepatic vein. In some examples, the injection is administered at a hepatic artery. In some examples, the condition is a liver-associated disease or disorder. In some examples, the condition is a liver failure. In some examples, the liver-associated disease or disorder comprises alagille syndrome, alpha 1 anti-trypsin deficiency, autoimmune hepatitis, benign liver tumors, biliary atresia, cirrhosis, cystic disease of the liver, fatty liver disease including alcohol-related liver disease and non-alcohol fatty liver disease (NAFLD), galactosemia, gallstones, Gilbert's Syndrome, hemochromatosis, liver cysts, liver cancer, liver disease in pregnancy (optionally, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, preeclampsia, or HELLP Syndrome (hemolysis, elevated liver tests, low platelets)), neonatal hepatitis, primary billary cirrhosis, primary sclerosing cholangitis, porphyria, Reye's Syndrome, sarcoidosis, toxic hepatitis, type 1 glocogen storage disease, tyrosinemia, viral hepatitis, Wilson disease, or any combination thereof.

[0013] In another example, disclosed herein is a use of a composition comprising a hepatocyte herein for the production of therapeutic proteins. In some examples, the therapeutic proteins comprise major plasma proteins such as human serum albumin, soluble plasma fibronectin, α-fetoprotein, C-reactive protein, and several globulins; proteins involved in hemostasis and fibrinolysis such as coagulation factors involved in the coagulation cascade, α2-macroglobulin, α1-antitrypsin, antithrombin III, protein S, protein C, plasminogen, α2-antiplasmin, and complement component 3; carrier proteins such as albumin, ceruloplasmin, transcortin, haptoglobin, hemopexin, IGF binding rotein, major urinary proteins, retinol binding protein, sex hormone-binding globulin, transthyretin, transferrin, and Vitamin D-binding protein; hormones such as insulin-like growth factor 1, thrombopoietin, hepcidin, and betatrophin; prohormones such as angiotensinogen; or apolipoproteins.

[0014] Also disclosed herein is a use of a composition comprising a hepatocyte herein for liver regeneration. In some examples, the liver regeneration is an *ex vivo* liver regeneration. In some examples, the *ex vivo* liver regeneration is a bioprinting method. In some examples, the bioprinting method is a 3 dimensional bioprinting method

[0015] In one example, disclosed herein is a use of a composition comprising a hepatocyte herein for bioprinting. In some examples, the bioprinting is a 3 dimensional bioprinting.

[0016] Also disclosed herein is a use of the hepatocyte herein for tissue scaffold generation. In some examples, the tissue scaffold is a 3 dimensional tissue scaffold.

[0017] In one example, disclosed herein is a use of a composition comprising a hepatocyte herein for gene therapy. In some examples, the gene therapy is an *ex vivo* gene therapy.

[0018] In another example, disclosed herein is an artificial tissue generated from hepatocytes herein. In some examples, the tissue is three-dimensional. In some examples, the issue is vascularized.

[0019] Also disclosed herein is an artificial organ generated from hepatocytes herein.

[0020] In some examples, a hepatocyte, tissue, or organ disclosed herein produces AFP, ALB, alpha-1-antitrypsin, glucose, or glycogen. In some examples, the hepatocyte, tissue, or organ metabolizes a lipid, cholesterol, or carbohydrate. In some examples, the hepatocyte, tissue, or organ metabolizes a pharmaceutical drug or toxic substance. In some examples, the hepatocyte, tissue, or organ uptakes ammonia or bile acid.

[0021] In some examples, a hepatocyte herein has comparable phenotypic (e.g., immunophenotypic) properties as a primary hepatocyte. In some examples, a hepatocyte herein has comparable morphologic properties as a primary hepatocyte. In some examples, a hepatocyte herein has comparable functional properties as a primary hepatocyte.

[0022] In some examples, a hepatocyte, tissue, or organ disclosed herein has one or more functions of: synthesis of fatty acids, triglycerides, cholesterol, bile salts, or phospholipids; detoxification, modification, and excretion of exogenous or endogenous compounds (e.g., drug, insecticide, steroid, ammonia, heavy metal, or toxin); carbohydrate metabolism; synthesis of proteins (e.g., serum albumin, fibrinogen, lipoprotein, apoprotein, ceruloplasmin, transferrin, complement, or glycoprotein); protein storage; or formation or secretion of bile. In some instances, a hepatocyte can be a hepatic progenitor cell (e.g., hepatocyte-like cell) or a hepatocyte derived from a stem cell; a hepatic stem cell; or a primary hepatocyte (e.g., are or comparable to freshly isolated or uncultured, cryopreserved hepatocytes obtained from a liver).

[0023] In some examples, a trophoblast stem cell disclosed herein is derived from an ectopic pregnancy mass (e.g., tubal). In some examples, the method of isolating a trophoblast stem cell herein comprises the steps of: obtaining trophoblastic villi from an ectopic pregnancy mass (e.g., tubal); collecting cells from the trophoblastic villi; and culturing the collected cells in a culture medium to obtain the isolated trophoblast stem cell. In some examples, the method further comprises cutting the trophoblastic villi into pieces. In some examples, the method further comprises treating the trophoblastic villi with an enzyme. In some examples, the human trophoblast stem cell is genetically modified to introduce a mutation into the cell. In some

examples, the pregnant mass is obtained in an unruptured manner. In some examples, the pregnant mass is at a gestational age of no older than 7 or 8 weeks. In some examples, the culture medium is free of a feeder layer. In some examples, the method further comprises the steps of: forming embryonic bodies (EBs) in the culture medium; treating the EBs with an enzyme; and collecting cells from the enzyme-treated EBs to obtain the isolated human trophoblast stem cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Figures 1A-1F illustrate immunoreactive markers of DE lineages. Figures 1A-1D show immunocytochemistry to detect FOXA2 and SOX17 (Figure 1A), GSC (Figure 1B), and MIXL1 (Figure 1C) at 4 hr of bFGF induction compared to the control in hTS cells. Figure 1D shows western blots in time course of DE-related transcription factors including GSC, Brachyury, MIXL1, SOX17, and FOXA2 at initial 8 hr of bFGF induction. Error bars indicate standard deviation (SD) of mean. N = 3, *: $p < 0.05$ as statistic significant. Figure 1E shows colocalization of hepatocyte-associated markers showing AFP and albumin (upper panel) and ABCC2 and BSEP (lower panel) at day-4 after induction. Figure 1F shows a two-step regimen for hepatocyte-like cell differentiation showing various hepatocyte-specific markers by Western blotting assay in hTS cells. α-tubulin as loading control.

[0025] Figures 2A-2M illustrates regulatory Molecular Mechanisms for DE Specification. Figure 2A indicates miR-124a analysis (ChIP-qPCR) which identifies that CREB1 targets at three sites of promoter of miR-124a mRNA. A schematic drawing of the consensus miR-124a binding sites (upper panel). C: as no-antibody control. Error bars indicate SD of 3 replicates. Figure 2B illustrates that knockdown of CREB1 reduces the bFGF-induced miR-124a expression by immunoblotting assay. Error bars indicate SD of 3 replicates. *: $p < 0.05$. Figure 2C shows bFGF induced expression of phosphor(p)-CREB1 and miRNA-124a during 8 hr induction by qPCR assay. Error bars indicate SD of 4 replicates. Figure 2D shows ChIP-qPCR of Smad4 which identifies that miR-124a represses Smad4 expression via targeting two sites of the promoter by luciferase reporter assay. The schematic drawing of the consensus miR-124a binding sites (upper panel). Empty vector as control; pSmad4 indicating Smad4 plasmid, Error bars indicate SD of 3 replicates. *: $p < 0.05$. Figure 2E shows the effects of bFGF, miR-124a, and anti-miR-124a antibody on the expression of DE-related transcription factors by Western blots. β-actin was used as loading control. Figure 2F shows the knockdown of Smad4 using shRNAs represses expression of Smad4 and MIXL1 by Western blots. β-actin was used as loading control. Figure 2G and Figure 2H show ChIP-qPCR assays which identify the inhibitory GSK3β by miR-124a by luciferase report assays at 4 hr induction (Figure 2G); while qPCR assay showed an inhibitory FOXA2 by β-catenin (Figure 2H). A schematic drawing of the consensus