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.

BRIEF SUMMARY

[0003] In one of many aspects, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the steroid and the cytokine are added simultaneously or sequentially into the conditioned medium.

[0003a] In one aspect, disclosed herein is a method of inducing a TS cell to differentiate into an induced hepatocyte in vitro, comprising: contacting the TS cell with a FGF that binds to FGFR1 to induce formation of a definitive endoderm (DE) cell, and contacting the DE cell with

a medium to induce differentiation into an induced hepatocyte, wherein the medium comprises a FGF that binds to FGFR1, a glucocorticoid steroid, and an interleukin 6 group cytokine.

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

CLAIMS

WHAT IS CLAIMED IS:

1. 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 fibroblast growth factor (FGF) that binds to FGFR1 to induce formation of a definitive endoderm (DE) cell, and contacting the DE cell with a medium to induce differentiation into an induced hepatocyte,

    wherein the medium comprises a FGF that binds to FGFR1, a glucocorticoid steroid, and an interleukin 6 group cytokine.

2. The method of claim 1, wherein the induced hepatocyte is a hepatic progenitor cell.
3. The method of claim 1, wherein the TS cell is a human TS cell.
4. The method of claim 1, wherein the glucocorticoid steroid is dexamethasone.
5. The method of claim 1, wherein the interleukin 6 group cytokine is oncostatin M.
6. The method of claim 5, wherein the oncostatin M is a human oncostatin M.
7. The method of claim 6, wherein the human oncostatin M is a recombinant human oncostatin M.
8. The method of claim 1, wherein the medium further comprises a bone morphogenetic protein (BMP).
9. The method of claim 8, wherein the BMP is BMP4.
10. The method of claim 1, wherein the medium further comprises a hepatic growth factor (HGF).
11. The method of claim 1, wherein the induced hepatocyte is immune privileged.
12. The method of claim 1, wherein the induced hepatocytes recruit $CD4^{+}Foxp3^{+}$ Treg cells.
13. The method of claim 1, wherein the induced hepatocytes form tissue of a 3-dimensional structure.
14. The method of claim 1, wherein the induced hepatocytes cluster or aggregate.
15. The method of claim 1, wherein the induced hepatocytes form a crescent cell mass.
16. The method of claim 1, wherein the induced hepatocytes comprise a peripheral compartment and a central compartment.
17. The method of claim 16, wherein the induced hepatocytes distribute irregularly along extracellular matrix (ECM) beyond basement membrane in the peripheral compartment.

18. The method of claim 16, wherein the induced hepatocytes distribute from basal towards central areas in the central compartment.

19. The method of claim 1, wherein the induced hepatocyte expresses one or more markers selected from the group consisting of transforming growth factor beta 1 (TGFβ1), human leukocyte antigen G (HLA-G), human cytoplasmic marker stem 121 (stem 121), mast/stem cell growth factor receptor C-kit (C-kit), cytokeratin 19 (CK19), cytokeratin 18 (CK18), albumin (ALB), alpha-fetoprotein (AFP), betatrophin, alcohol dehydrogenase-1 (ADH1), apolipoprotein F (APOF), 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), and any combination thereof.

20. The method of claim 1, wherein the trophoblast stem cell is contacted with the FGF selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF8, FGF10, FGF17, FGF19, FGF20, FGF21, FGF22, and FGF23.

21. The method of claim 20, wherein the FGF is FGF2 (bFGF).

22. The method of claim 20, wherein the FGF is FGF1.

23. The method of claim 1, wherein the glucocorticoid steroid is selected from the group consisting of dexamethasone, betamethasone, budesonide, cortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone.

24. The method of claim 1, wherein the interleukin 6 group cytokine is selected from the group consisting of interleukin-6, oncostatin M, interleukin-11, leukemia inhibitory factor (LIF), ciliary neurotropic factor (CNTF), cardiotrophin-1(CT-1), and cardiotrophin-like cytokine (CLC).

25. The method of claim 1, wherein the medium comprises:
the FGF selected from the group consisting of: FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF8, FGF10, FGF17, FGF19, FGF20, FGF21, FGF22, and FGF23;
the glucocorticoid steroid selected from the group consisting of: dexamethasone, betamethasone, budesonide, cortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone; and
the interleukin 6 group cytokine selected from the group consisting of: interleukin-6, oncostatin M, interleukin-11, leukemia inhibitory factor (LIF), ciliary neurotropic factor (CNTF), cardiotrophin-1 (CT-1), and cardiotrophin-like cytokine (CLC).

26. The method of claim 25, wherein the medium comprises: FGF2; dexamethasone; and oncostatin M.