METHODS AND COMPOSITIONS FOR STEM CELL DIFFERENTIATION

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional application no. 63/352,529, filed on June 15, 2022, the entirety of which is hereby incorporated by reference herein.

BACKGROUND

[0002] Hepatocytes serve an important role in the liver: they can be involved in the synthesis or storage of proteins; transformation of carbohydrates; the synthesis of cholesterol, bile salts, or phospholipids; and detoxification. Therapies to improve certain functions of the liver may involve the introduction or modulation of hepatocytes or hepatic cells. Stem cells can be programmed into various phenotypes via the introduction of one or more transcription factors, or one or more molecules that modulate transcription or transcription factors. For example, stem cells can be programmed into hepatocytes or hepatic cells.

SUMMARY

[0002a] In one aspect, provided herein is a pluripotent stem cell (PSC) engineered to express a nucleic acid comprising one or more transcription factors, wherein the PSC expresses at least three hepatocyte markers in a time period of 96 hours or less after induction of the one or more transcription factors.

[0002b] In another aspect, provided herein is a method of generating a population of hepatocytes or hepatic cells comprising: (a) providing one or more pluripotent stem cells (PSCs); (b) delivering to said one or more PSCs at least one nucleic acid comprising one or more transcription factors; (c) generating said population of hepatocytes or hepatic cells from said one or more PSCs, wherein the PSCs express at least three hepatocyte markers in a time period of 96 hours or less after induction of the one or more transcription factors.

[0002c] In another aspect, provided herein is an exogenous expression cassette that induces differentiation of a pluripotent stem cell (PSC) into a hepatocyte or a hepatic cell, wherein the PSC expresses at least three hepatocyte markers in a time period of 96 hours or less after induction of the exogenous expression cassette comprising one or more transcription factor genes.

[0002d] In another aspect, provided herein is a pluripotent stem cell (PSC) comprising a nucleic acid comprising an open reading frame encoding one or more transcription factors, one or more transcription factors, or activator of transcription of the open reading frame encoding one or more transcription factors, wherein said one or more transcription factors comprise: FOXA1, HNF1A, FOXA2, CEBPA, ONECUT1/HNF6, HNF4A, and RBPJ.

[0002e] In another aspect, provided herein is a pluripotent stem cell (PSC) comprising a nucleic acid comprising an open reading frame encoding one or more transcription factors, one or more transcription factors, or activator of transcription of the open reading frame encoding one or more transcription factors, wherein said one or more transcription factors comprise: HNF4A, HNF1A, ONECUT1/HNF6, and FOXA1.

[0002f] In another aspect, provided herein is a pluripotent stem cell (PSC) comprising a nucleic acid comprising an open reading frame encoding one or more transcription factors, one or more transcription factors, or activator of transcription of the open reading frame encoding one or more transcription factors, wherein said one or more transcription factors comprise: FOXA1, HNF1A, CEBPA, ONECUT1/HNF6, and HNF4A.

[0002g] In another aspect, provided herein is a pluripotent stem cell (PSC) comprising a nucleic acid comprising an open reading frame encoding one or more transcription factors, one or more transcription factors, or activator of transcription of the open reading frame encoding one or more transcription factors, wherein said one or more transcription factors comprise: CEBPA, FOXA1, HNF1A, HNF4A, ONECUT1/HNF6, and RBPJ.

[0002h] In another aspect, provided herein is a pluripotent stem cell (PSC) comprising a nucleic acid comprising an open reading frame encoding one or more transcription factors, one or more transcription factors, or activator of transcription of the open reading frame encoding one or more transcription factors, wherein said one or more transcription factors comprise: CEBPA, FOXA1, HNF1A, and HNF4A.

[0002i] In another aspect, provided herein is a kit for inducing differentiation of a pluripotent stem cell (PSC) into a hepatocyte or a hepatic cell, the kit comprising one or more transcription factors, wherein the PSC expresses at least three hepatocyte markers in a time period of 96 hours or less after induction of the one or more transcription factors.

[0003] An aspect of the present disclosure is a pluripotent stem cell (PSC) engineered to express a nucleic acid comprising an open reading frame encoding one or more transcription factors, one or more transcription factors, or activator of transcription of the open reading frame encoding one or more transcription factors, wherein the one or more transcription factors comprising: one or more hepatocyte nuclear factor (“HNF”) family members; one or more forkhead box (“FOX”)

family members; and one or more additional transcription factors comprising FOS, JUN, FOX, PROX, GATA, HHEX, HIF, HNF, MYC, CEBP, NFE, NFI, NR, RBPJ, RXR, SMAD, SOX, SPI, TBX, TP, PPAR, SALL, EGR, ONECUT, SKI or HLF family members, or one or more additional transcription factors from Table 12. In some embodiments, the one or more transcription factors comprises 2 or more HNF family members. In some embodiments, the one or more transcription factors comprises 3 or more HNF family members. In some embodiments, the one or more additional transcription factors comprises CEBP, and the one or more transcription factors further comprises FOS, JUN, FOX, PROX, GATA, HHEX, HIF, HNF, MYC, CEBP, NFE, NFI, NR, RBPJ, RXR, SMAD, SOX, SPI, TBX, TP, PPAR, SALL, EGR, ONECUT, SKI or HLF family members, or one or more additional transcription factors from Table 12. In some embodiments, the one or more additional transcription factors comprises