This invention relates to a process for the high efficiency *in vitro* induction of human iPS cells into hepatic progenitor cells and hepatocytes. This may be useful, for example, in producing hepatocytes for cell-based therapies or disease modelling.

Summary

In one aspect, the present disclosure refers to a method for inducing hepatic differentiation comprising;

(i) providing a population of induced pluripotent stem (iPS) cells, optionally human iPS cells,

(ii) culturing the population to produce a population of anterior definitive endoderm (ADE) cells,

wherein said culturing comprises;

(a) culturing the population in a endoderm induction medium; wherein the endoderm induction medium is a chemically defined medium which comprises fibroblast growth factor; a TGFβ ligand which stimulates SMAD2 and SMAD3 mediated signalling pathways; bone morphogenic protein; a phosphatidylinositol 3-kinase (PI3K) inhibitor and a glycogen synthase kinase 3β (GSK3β) inhibitor;

(b) further culturing the population in a second endoderm induction medium, wherein the second endoderm induction medium is a chemically defined medium which comprises fibroblast growth factor; a TGFβ ligand which stimulates SMAD2 and SMAD3 mediated signalling pathways; bone morphogenic protein; and a phosphatidylinositol 3-kinase (PI3K) inhibitor and lacks glycogen synthase kinase 3β (GSK3β) inhibitor; and

(c) further culturing the population in ADE induction medium which comprises a TGFβ ligand which stimulates SMAD2 and SMAD3 signalling pathways and fibroblast growth factor to produce the population of anterior definitive endoderm (ADE) cells, and,

(iii) culturing the population of ADE cells in a hepatic induction medium to produce a population of hepatic progenitor cells,

wherein the hepatic induction medium is a chemically defined medium which comprises a TGFβ ligand which stimulates SMAD2 and SMAD3 mediated signalling pathways.

In another aspect, the present disclosure refers to a population of isolated hepatocytes produced by a method as disclosed herein wherein said hepatocytes display the phenotype of an inherited metabolic disorder (IMD) of the liver, and;
wherein the hepatocytes express the mature adult hepatocyte markers albumin (ALB) and α-1-antitrypsin (AAT) and the foetal hepatocyte markers alpha-Fetoprotein (AFP) and CYP3A7.

In another aspect, the present disclosure refers to a method of screening a compound comprising;

contacting a population of isolated hepatocytes as disclosed herein with a test compound, and;
determining the effect of the test compound on said hepatocytes and/or the effect of said hepatocytes on the test compound.

In yet another aspect, the present disclosure refers to a method of screening for a compound useful in the treatment of a liver disease comprising;

contacting a population of isolated hepatocytes as disclosed herein with a test compound, and;
determining the effect of the test compound on said hepatocytes.

Detailed Description

An aspect of the invention provides a method for inducing hepatic differentiation comprising;

(i) providing a population of induced pluripotent stem (iPS) cells,

(ii) culturing the population in a endoderm induction medium to produce a population of anterior definitive endoderm (ADE) cells,

wherein the endoderm induction medium is a chemically defined medium which has fibroblast growth factor activity and which stimulates SMAD2 and SMAD3 mediated signalling pathways and SMAD1, SMAD5 and SMAD9

mediated signalling pathways and inhibits phosphatidylinositol 3-kinase (PI3K) and glycogen synthase kinase 3β (GSK3β); and,

(iii) culturing the population of ADE cells in a hepatic induction medium to produce a population of hepatic progenitor cells,

wherein the hepatic induction medium is a chemically defined medium which stimulates SMAD2 and SMAD3 mediated signalling pathways.

The method may further comprise;

(iv) culturing the population of hepatic progenitor cells in a hepatic maturation medium to produce a population of hepatocytes.

Preferably the iPS cells are human iPS cells.

iPS cells are pluripotent cells which are derived from non-pluripotent, fully differentiated ancestor cells. Suitable ancestor cells include adult fibroblasts and peripheral blood cells. Ancestor cells are typically reprogrammed by the introduction of pluripotency genes or proteins, such as Oct4, Sox2 and Sox1 into the cell. The genes or proteins may be introduced into the differentiated cells by any suitable technique, including plasmid or more preferably, viral transfection or direct protein delivery. Other genes, for example Kif genes, such as Kif-1, -2, -4 and -5; Myc genes such as C-myc, L-myc and N-myc; nanog; and Lin28 may also be introduced into the cell to increase induction efficiency. Following

Claims:

1. A method for inducing hepatic differentiation comprising;

(i) providing a population of induced pluripotent stem (iPS) cells, optionally human iPS cells,

(ii) culturing the population to produce a population of anterior definitive endoderm (ADE) cells,

wherein said culturing comprises;

(a) culturing the population in a endoderm induction medium; wherein the endoderm induction medium is a chemically defined medium which comprises fibroblast growth factor; a TGFβ ligand which stimulates SMAD2 and SMAD3 mediated signalling pathways; bone morphogenic protein; a phosphatidylinositol 3-kinase (PI3K) inhibitor and a glycogen synthase kinase 3β (GSK3β) inhibitor;

(b) further culturing the population in a second endoderm induction medium, wherein the second endoderm induction medium is a chemically defined medium which comprises fibroblast growth factor; a TGFβ ligand which stimulates SMAD2 and SMAD3 mediated signalling pathways; bone morphogenic protein; and a phosphatidylinositol 3-kinase (PI3K) inhibitor and lacks glycogen synthase kinase 3β (GSK3β) inhibitor; and

(c) further culturing the population in ADE induction medium which comprises a TGFβ ligand which stimulates SMAD2 and SMAD3 signalling pathways and fibroblast growth factor to produce the population of anterior definitive endoderm (ADE) cells, and,

(iii) culturing the population of ADE cells in a hepatic induction medium to produce a population of hepatic progenitor cells,

wherein the hepatic induction medium is a chemically defined medium which comprises a TGFβ ligand which stimulates SMAD2 and SMAD3 mediated signalling pathways.

2. A method according to claim 1 wherein the TGFβ ligand is activin, the PI3K inhibitor is LY294002 and/or the GSK3β inhibitor is CHIR99021.

3. A method according to any one of the preceding claims further comprising;

(iv) culturing the population of hepatic progenitor cells in a hepatic maturation medium to produce a population of hepatocytes.

4. A method according to any one of the preceding claims comprising expanding culturing, maintaining or storing the population of hepatic progenitor cells or hepatocytes.

5. A method according to any one of the preceding claims comprising admixing the population of hepatic progenitor cells or hepatocytes with a pharmaceutically acceptable excipient.

6. A method according to any one of the preceding claims wherein the iPS cells are derived from cells obtained from an individual with damaged or dysfunctional liver tissue and the hepatocytes in the population display a non-disease associated phenotype.

7. A method according to claim 6 wherein a liver disease associated genetic mutation or defect in the iPS cells is corrected, such that said hepatocytes display a non-disease associated phenotype.

8. A method according to claim 7 wherein the genetic mutation or defect is a Glu342Lys mutation in alpha 1 antitrypsin (A1AT) and the liver disease is alpha 1 antitrypsin deficiency.

9. A method according to any one of claims 1 to 6 wherein the iPS cells are derived from cells obtained from an individual with a liver disease and the produced hepatic precursor cells or produced hepatocytes in the population display a disease phenotype.

10. A method according to claim 9 wherein the liver disease is an inherited metabolic disorder (IMD), optionally an IMD selected from the group consisting of Alpha 1 Antitrypsin deficiency, a Glycogen Storage Disease, Familial Hypercholesterolemia, Hereditary

Tyrosinaemia, Crigler Najjar syndrome, ornithine transcarbamylase deficiency, factor IX deficiency, haemochromatosis, Wilson's disease, Dubin-Johnson syndrome, familial amyloidosis, and Refsum's disease.

11. A population of isolated hepatocytes produced by a method according to claim 10 wherein said hepatocytes display the phenotype of an inherited metabolic disorder (IMD) of the liver, and;

wherein the hepatocytes express the mature adult hepatocyte markers albumin (ALB) and α-1-antitrypsin (AAT) and the foetal hepatocyte markers alpha-Fetoprotein (AFP) and CYP3A7.

12. A method of screening a compound comprising;

contacting a population of isolated hepatocytes according to claim 11 with a test compound, and;

determining the effect of the test compound on said hepatocytes and/or the effect of said hepatocytes on the test compound.

13. A method of screening for a compound useful in the treatment of a liver disease comprising;

contacting a population of isolated hepatocytes according to claim 11 with a test compound, and;

determining the effect of the test compound on said hepatocytes.

14. A method according to claim 13 wherein the effect of the test compound on one or more disease pathologies in the hepatocytes is determined,

wherein a decrease or amelioration of one or more disease pathologies in the hepatocytes in the presence, relative to the absence of test compound is indicative that the test compound may be useful in the treatment of a liver disease.