circumvent this, by growth arrest of cells by adding 1-2% dimethyl sulfoxide (DMSO, a polar aprotic solvent) to the cell culture medium (Sainz et al. (2006) J Virol 80: 10253-10257), resulting in the induction of expression of hepatocyte-specific genes.

[004] Freshly isolated primary human hepatocytes are logically a more representative in vitro model to study HCV infectivity. However, the amount of virus produced in these cells is low (typcially less than 10^3 RNA copies/ml), and for long term experiments (more than a few days) these cells have to be co-cultured with other cell types (Banaudha et al (2010) Hepatology, 51: 1922–1932; Ploss et al. Proc. Natl. Acad. Sciences 2010 vol. 107 no. 7 3141-3145). Primary hepatocytes are thus not suitable for large scale virus production.

[005] HCV viral titers have been achieved in HuH-7 or HuH-7-derived cells of approximately 10^6 to 10^7 RNA copies per ml (about 1 virus per cell). However, these viral titers are generally too low to provide for commercial scale production of viral particles. Additionally, infection in HuH7.5 cells has only been possible with an atypical HCV variant, JFH-1.

[006] There is a need in the field for a culture system that can serve as an in vitro model of primary human hepatocytes.

SUMMARY

[006a] In a first aspect of the present disclosure, there is provided a method of producing a cell culture comprising cells having a primary human hepatocyte phenotype, the method comprising:

culturing a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days

wherein said culturing induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype.

[006b] In a second aspect of the present disclosure, there is provided a cell culture comprising cells that are differentiated progeny of the hHCC cell line and having a phenotype of a human primary hepatocyte, wherein the cells are produced by the method of the first aspect.

[006c] In a third aspect of the present disclosure, there is provided a cell culture comprising:

cells having a phenotype of a human primary hepatocyte, wherein the cells are the differentiated progeny of an hHCC cell line; and

a culture medium comprising human serum.

[006d] In a fourth aspect of the present disclosure, there is provided a method for assessing an effect of a candidate agent on a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of the ~~third~~ second aspect with a candidate agent; and

assaying for the presence ~~of~~ or absence of an effect of the candidate agent on a phenotype of the cell~~s having a phenotype of a human primary hepatocyte~~.

[006e] In a fifth aspect of the present disclosure, there is provided a method for assessing metabolism of an agent by a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of the ~~third~~ second aspect with an agent; and

assaying for the presence of absence of a metabolite of the agent and/or the agent.

[006f] In a sixth aspect of the present disclosure, there is provided a method for assessing toxicity of an agent on a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of the ~~third~~ second aspect with an agent; and

assaying for the presence of absence of a change in a phenotype of the cells which is indicative of toxicity of the agent for the cell.

[006g] In a seventh aspect of the present disclosure, there is provided a method of producing viral particles, the method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days.

wherein said incubating induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype;

introducing a genome of a hepatotrophic virus into at least one of the hHCC cell line or the cell having primary human hepatocyte phenotype; and

maintaining the cell culture under conditions suitable for production of viral particles.

[006h] In an eighth aspect of the present disclosure, there is provided a virally-infected cell culture comprising cells produced by a method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days, wherein said culturing

wherein a decrease in viral particle production in the presence of the sample as compared to the absence of the sample indicates the sample contains an antibody having antiviral activity.

[006k] In an eleventh aspect of the present disclosure, there is provided a method for screening for a candidate agent for the treatment of a lipoprotein mediated disease, the method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for at least ~~3~~ 11 days, wherein said incubating induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype;

contacting the cell culture with the candidate agent; and

assaying the cell culture for the presence or absence of an effect of the candidate agent on the levels of lipoprotein secreted by the differentiated hHCC cell line as compared to a control sample;

wherein an effect of the candidate agent on the levels of lipoprotein secreted by the differentiated hHCC cell line indicates that the candidate agent can be used for the treatment of the lipoprotein mediated disease.

~~[006l] In a twelfth aspect of the present disclosure, there is provided a method of infecting a cultured hepatocytic cell with a hepatotrophic microorganism, the method comprising:~~

~~contacting a cultured hepatocyte cell with an infectious hepatotrophic microorganism in a culture medium comprising serum depleted of LDL-receptor binding lipoproteins;~~

~~wherein said contacting is for a time sufficient to provide for infection of the cultured hepatocyte cell with the hepatotrophic microorganism.~~

[007] The present disclosure provides methods and compositions relating to in vitro cultures of human hepatocyte cell lines which exhibit a primary human hepatocyte phenotype. Such cell lines are susceptible to infection by a hepatotrophic virus, such as HCVor HBV, and support both viral replication and high levels of viral particle production. Such in vitro cultures find use in production and study of hepatotrophic virus, as well as methods of screening (e.g., for antiviral drugs, assessing drug metabolism), and study of primary human hepatocytes.

[008] The present disclosure provides methods of producing a cell culture comprising cells having a primary human hepatocyte phenotype, which method comprises culturing a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days, wherein said culturing induces differentiation of the hHCC cell line into a

CLAIMS

What is claimed is:

1. A method of producing a cell culture comprising cells having a primary human hepatocyte phenotype, the method comprising:

culturing a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for more than 11 days

wherein said culturing induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype.

2. The method of claim 1, wherein the culture medium comprises about 1% to 20% human serum.

3. The method of claim 1, wherein the culture medium comprises about 2% to 10% human serum.

4. The method of any one of claims 1-3, wherein the hHCC cell line is a HuH-7 or HuH-7-derived cell line.

5. The method of any one of claims 1-4, wherein said culturing is without subculturing after 10 days of culturing in the culture medium comprising human serum.

6. A cell culture comprising cells that are differentiated progeny of the hHCC cell line and having a phenotype of a human primary hepatocyte, wherein the cells are produced by the method of any one of claims 1-5.

7. A cell culture comprising:

cells having a phenotype of a human primary hepatocyte, wherein the cells are the differentiated progeny of an hHCC cell line; and

a culture medium comprising human serum.

8. A method for assessing an effect of a candidate agent on a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of claim ~~7~~ 6 with a candidate agent; and

assaying for the presence ~~of~~ or absence of an effect of the candidate agent on a phenotype of the cells ~~having a phenotype of a human primary hepatocyte~~.

9. The method of claim 8, wherein said assaying is for an effect of the candidate agent on lipid metabolism by the cell having a phenotype of a primary human hepatocyte.

10. The method of claim 8, wherein said assaying is for an effect of the candidate agent on very low density lipoprotein (VLDL), low density lipoprotein (LDL), and/or high density lipoprotein (HDL) secretion by the cells ~~having a phenotype of a primary human hepatocyte~~.

11. A method for assessing metabolism of an agent by a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of claim ~~7~~ 6 with an agent; and

assaying for the presence of absence of a metabolite of the agent and/or the agent.

12. The method of claim 11, wherein the agent is a drug.

13. A method for assessing toxicity of an agent on a cell having a phenotype of a human primary hepatocyte, the method comprising:

contacting the cell culture of claim ~~7~~ 6 with an agent; and

assaying for the presence of absence of a change in a phenotype of the cells which is indicative of toxicity of the agent for the cell.

14. The method of claim 13, wherein the phenotype is an increase in transaminase in culture medium.

15. The method of claim 13, wherein the phenotype is an increase in a marker of cell death.

31. A method for screening for a candidate agent for the treatment of a lipoprotein mediated disease, the method comprising:

incubating a cell culture comprising a human hepatocellular carcinoma (hHCC) cell line in a culture medium comprising human serum for at least ~~3~~ 11 days, wherein said incubating induces differentiation of the hHCC cell line into a cell having a primary human hepatocyte phenotype;

contacting the cell culture with the candidate agent; and

assaying the cell culture for the presence or absence of an effect of the candidate agent on the levels of lipoprotein secreted by the differentiated hHCC cell line as compared to a control sample;

wherein an effect of the candidate agent on the levels of lipoprotein secreted by the differentiated hHCC cell line indicates that the candidate agent can be used for the treatment of the lipoprotein mediated disease.

32. The method of claim 31, wherein the cell culture is assayed for the presence or absence of an effect of the candidate agent on the levels of very low density lipoprotein (VLDL), low density lipoprotein (LDL), and/or high density lipoprotein (HDL) in the culture medium as compared to a control sample.

33 The method of claim 31, wherein the method is for the screening for a candidate agent for the treatment of atherosclerosis, and wherein a decrease in VLDL or LDL or an increase in HDL indicates that the candidate agent can be used for the treatment of atherosclerosis.

~~34. A method of infecting a cultured hepatocytic cell with a hepatotrophic microorganism, the method comprising:~~

~~contacting a cultured hepatocyte cell with an infectious hepatotrophic microorganism in a culture medium comprising serum depleted of LDL-receptor binding lipoproteins;~~

~~wherein said contacting is for a time sufficient to provide for infection of the cultured hepatocyte cell with the hepatotrophic microorganism.~~

~~35. The method of claim 34, wherein the cultured cells are primary hepatocytes or an immortalized hepatocyte cell line.~~

~~36. The method of claim 34, wherein the cultured cells are differentiated hHCC cells.~~

~~37. The method of claim 34, wherein the serum depleted of LDL-receptor binding lipoproteins is human serum depleted of LDL-receptor binding lipoproteins or fetal bovine serum depleted of LDL-receptor binding lipoproteins.~~

~~38. The method of any of claims 34-37, wherein the infectious hepatotrophic microorganism is a hepatotrophic virus.~~

~~39. The method of claim 38, wherein the hepatotrophic virus is hepatitis C virus or hepatitis B virus.~~

~~40. The method of claim 38 or 39, wherein the hepatotrophic virus is a clinical isolate.~~