[Frontiers in Bioscience, Scholar, 8, 97-105, January 1, 2016]

Modeling of HBV and HCV hepatitis with Hepatocyte-like cells

Abderrahmane Bengrine[1], Etienne Brochot[2,3], Marie Louchet[1,2], Yves Edouard Herpe[1] Gilles Duverlie[1,2,3]

[1]Biobanque de Picardie, CHU Amiens-Picardie, Amiens cedex 1 France, [2]Virology Research Unit, EA 4294, Jules Verne University of Picardie, Amiens, France, [3]Department of Virology, CBH, CHU Amiens-Picardie, Amiens cedex 1 France

TABLE OF CONTENTS

1. ABSTRACT

Chronic liver diseases caused by either hepatitis B or C viruses are a major health problem around the world. Despite major advances accomplished in recent years in understanding the physiology of both viruses using *in vitro* and/or *in vivo* models, there is no vaccine for HCV available. Moreover, susceptibility to acute and chronic infection and the response to treatments are different between HBV or HCV infected patients. Crucial information can be collected using a robust cell model that permits the culture of clinical isolates along with the investigation of the virus-host interaction. The recent progress in the field of cell reprogramming and differentiation has opened new opportunities in viral hepatitis research raising the hopes of developing new improved therapeutics. In this review, we discuss current models for hepatitis B and C studies and their limitations, and also the iPSC model, and its relevance to the viral host cell interactions.

2. INTRODUCTION

Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) are a major public health problem with approximately 520 million people infected with one of these viruses worldwide (170 and 350 million respectively). A significant portion of the infected population will develop virus related complications such as cirrhosis and liver cancer (1, 2). In the case of HCV 15–45% of infected patients will clear the virus within 6 months without intervention, however the mechanism remains unknown (3). Understanding the relationship between the viruses and the host cell is hampered by the absence of viral culture models that support the infection and accurately reproduce what is really happening in the host cell. The gold standard cell model for studying liver infectious disease is the primary human hepatocyte (PHH). However, the rapid loss of the hepatic phenotype after isolation from the liver environment, the restricted availability and the variability among the donors are a major handicap. Moreover, PHH from different donors show variation in their permissiveness to HCV and HBV, suggesting a direct influence of host genetic factors on the infection. Over the past decade the reprogramming of somatic cells to induced pluripotent stem cells (iPSC) and their differentiation into hepatocyte-like cells (HLC) started to be considered a promising alternative to study liver aetiologies in general (4). HLCs are now considered a solution to circumvent the limited availability of healthy PHH and they can provide a system in which the influence of genetic factors on viral infections can be investigated.

Reprogramming of somatic cells into iPSC started with the pioneering work of Yamanaka and colleagues (5) and since then a growing number of laboratories have joined the field and reported new reprogramming technics from different somatic cells (6). Using embryology principles to differentiate pluripotent stem cells to hepatocytes, numerous scientific publications reported new methods to obtain HLCs from iPSC (7-12). The predisposition of human iPSC to differentiate to host-specific adult cells is considered a major hope for regenerative and personalized medicine, particularly to set up a model for physiopathological studies such as the interaction between HCV/HBV and the host hepatocyte. In this paper, we will review the existing cellular models for both HCV and HBV and discuss the contribution that iPSC differentiation into HLC may offer, to help understand, the physiopathology of these pathogens.

3. HCV AND HBV CULTURE MODELS

3.1. Model for studying HCV

The research on HCV infection has been hampered by the lack of relevant *in vitro* and *in vivo* models. The first robust models available for the *in vitro* studies were HCV replicons and pseudo-particles (13,14).

3.1.1. Huh-7 cell line and subclones

The discovery in 2005 of the unique strain of HCV JFH-1 of genotype 2a (isolated from a Japanese patient suffering from fulminant hepatitis) was considered a major step forward in understanding the virus life cycle. Huh-7 cell line and subclone Huh-7.5.1 (15-17) are the most widely used cell models to study HCV. Infected Huh-7 cells produce infectious progeny viruses (called HCVcc for cell culture) and thus recapitulate the entire life cycle of the virus. The production of infectious particles from chimeric viruses with intra- or inter-genotypic combinations has been also reported (18,19). The spontaneous emergence of adaptive mutations during successive passages in cell culture has improved production of highly infectious viral particles (20,21). Although major accomplishments have been obtained since the discovery of JFH-1, HCV research is still hampered by the incapacity of cultivating other HCV genotypes isolated from patient sera (22). It is possible that the difference in composition between HCV particles derived from patient sera (HCVser) and from HCVcc such as buoyant density and virion-associated serum products could be the reason behind this incapacity. Another explanation could be related to the nature of Huh-7 cell line. This transformed, rapidly dividing cell line is not the natural host of the virus. Huh-7 cells have several gene alterations such as mutations in the dsRNA sensor retinoic acid-inducible gene-I (RIG-I) (23) and deregulation of six major signalling pathways of interferon (IFN) (24).

3.1.2. Primary human hepatocytes

Primary human hepatocytes represent the most physiologically relevant cell model to investigate HCV infection. These differentiated cells allow replication of HCVcc, albeit with variable viral production (25,26), and lower permissiveness than that observed in Huh-7 cells with JFH-1. Moreover, PHHs have numerous limitations: (i) they originate from liver resections of patients with serious illnesses and different medical histories (27), (ii) poor quality of the cells and heterogeneity between batches (donor-dependent), (iii) low and unpredictable availability, (iv) absence of standard efficient cryopreservation protocol (28), (v) incapacity to proliferate *in vitro* and rapid loss of their phenotype and function. Finally, special care has to be taken not to co-purify nonparenchymal cells when investigating the IFN response to HCV infection, because they may be an alternative source for this cytokine (29).

3.2. Model for studying HBV

Like HCV, research on HBV has to overcome the lack of pertinent culture model capable of reproducing the events occurring *in vivo*. Several key issues related to HBV biology are still without answer including the identity of cellular receptor and the molecular mechanism governing the formation of the HBV micro-chromosome.

3.2.1. Cell lines

The hepatoma cell line HepaRG is permissive to HBV when it has been induced to differentiate by the DMSO (30). The lack of cccDNA amplification in these cells, plus the low viral yield and replication, render the comprehension of the live cycle of HBV very difficult. Moreover, the dependence of the infection upon the differentiation state of the cells is a source of conflicting results. Other human hepatoma cell lines are used to replicate the virus after being transfected. Huh-7 and HepG2 cell lines are sensitive to HBV produced in cell culture when they overexpress the sodium- taurocholate co-transporting polypeptide (NTCP) (31). Like HCV, the HBV present in patient serum is unable to propagate in cell culture, and the mechanism of inhibition is still undetermined. Moreover, hepatoma cell lines are known to carry many impaired cellular pathways like cell proliferation and innate immune response (32,33). Recently, a Chinese team has reported the development of a new hepatoma cell line HLCZ01 that supports the entire life cycles of both HBV and HCV and can be infected with different patient sera (34). The origin of the HLCZ01 cell line remains a handicap to consider that all the obtained results are reflecting exactly the cycle of the viruses in a cell with normal genetic background.

3.2.2. Primary human hepatocytes

The gold standard cells to study hepatic viral infection are the PHH. These cells are known to support the HBV infection but with a very low rate. Neither DMSO nor polyethylene glycol supplementation to cell culture media helped to increase HBV infection (35,36). The co-culture of PHH in micro-patterning with fibroblasts (MPCC) has been shown to improve the maintenance

of infection by HBV through the prevention of the rapid loss of hepatocytes phenotype and function (37). Although this system allows researchers to investigate virus pathophysiology, PHH co-culture with MPCC cannot overcome the difficulties related to variability and limitation of PHH sources.

3.3. Animal model for HCV and HBV

HCV and HBV have a narrow host range and exclusively infect immunocompetent hosts, human and chimpanzee. The use of the chimpanzees as animal model to investigate the physiopathology of these viruses is relatively expensive and raises some ethical concerns. Several transgenic mice harbouring one or more HCV or HBV genes have been very useful to study the impact of viral protein(s) in the development of liver diseases (38). In the case of HCV, the expression of human genes coding for different receptors used by the virus to enter hepatocytes and to replicate in a genetically modified mice has raised the hope that a permissive mouse model to HCV can be achieved (39).

Different attempts have been reported to reconstitute mouse liver using human hepatocytes to help preserving their functionality. Crossing between urokinase-type plasminogen activator (uPA) transgenic mice (40) deficient in fumaryl acetoacetate hydrolase (FAH) (41) and immunodeficient mice has been accomplished. The resulting progenitor has a liver that is susceptible to infection with HBV and HCV, as shown by detection of viral antigens in the chimeric liver of these animals. In addition, these mice when exposed to HCV, for instance, respond to antiviral treatment (42).

Taken together, the aforementioned models for HCV/HBV investigations have contributed significantly to the understanding of several aspects of the molecular physiology of these viruses but have failed to fully reproduce what is taking place in the natural host (human hepatocytes). Thus, an alternative source of healthy and fully functional hepatocytes is urgently needed. The optimization of the production of hepatocytes from human pluripotent stem cells (hESC) and induced pluripotent stem cells (iPSC) constitutes a promising alternative.

4. iPSC-DERIVED HEPATOCYTES: BENEFITS AND CONTRIBUTION FOR HEPATIC DISEASES

Human iPSC have the potential to greatly impact many areas of research and medicine for the following reasons: (i) they can be generated by reprogramming different somatic cells through a forced overexpression of key reprogramming factors, (ii) their capacity for self-renewal, and (iii) their ability to differentiate into cells found in the three germ layers (endoderm, mesoderm and ectoderm) constituting an organism. Thus, the differentiation of pluripotent stem cells to HLCs offers an alternative to primary human hepatocytes as an unlimited source of non-cancerous cells. HLCs are generated from human iPSC using the same approach as with pluripotent stem cells of diverse origins (11,43,44). The general strategy for iPSC induced hepatic differentiation is their exposition stepwise to different growth factors and cytokines mimicking the liver micro-environment during the development. Generation of clinically and scientifically valuable hepatocytes requires the establishment of procedures that efficiently and reproducibly differentiate iPSC into mature and functional HLCs.

Even though all HLCs resulting from the application of the existing procedures express different liver markers (albumin, alpha-1-antitrypsin, P450 cytochromes, miR-122 and proteins needed for HCV entry (Table 1), they still carry some foetal features, such as a lower expression of albumin and higher expression of alpha fetoprotein in comparison to freshly isolated PHHs (9-11). These findings suggest that the differentiation protocols need further optimization. Furthermore, different iPSC clones from the same patient respond differently to growth factors and cytokines induced differentiation (43). The existence of single nucleotide polymorphisms (SNPs) or sequence variations in cytokine receptors or in the downstream effectors that may influence this process constitute a plausible explanation. Moreover, the origin of iPSC may affect their propensity to generate HLCs as has been demonstrated for the same patient iPSC derived from peripheral blood or fibroblasts. This finding suggests that the genetic background of the donor has a strong impact on the hepatic differentiation (11). Despite great efforts to improve differentiation protocols to achieve these final stages of maturation, limited progress has been made so far. It is possible that the final stage of maturation is only possible in the *in vivo* microenvironment. In this case, the extracellular niche is the human liver or a device where the HLCs found themselves in 3D architecture, like spheroids for instance.

5. MODELING HCV AND HBV LIFE CYCLES USING iPSC DERIVED HLCs

Despite the limitations of the existing models, each one of them has contributed to the understanding and characterization of HCV/HBV molecular structures and helped to uncover their physiopathologies. In the case of HCV, the host factors are implicated in the history of the disease and modulate the response to treatment (polymorphism near the IL28B coding gene). The functions of these host factors are still poorly understood due to the lack of strong experimental systems (45). The expression of protein X (considered as crucial for the development of HBV related hepato-carcinoma (HCC) needs a non-cancer cellular model to be investigated (46).

Table 1. HCV and HBV infection of HLCs derived from hESC and iPSC

Number of hESC and iPSC clones	Protocol	Viral infection	Fetal futures of HLCs	Expression of liver-specific factors in HLCs	References
1 iPSC cell line	Adenovirus expressing SOX17, HEX or HNF-4 alpha and growth factors	Pseudo-particles: Entry (anti-CD81) Replication (IFN)	ND	P-450s (CYP2D6, CYP3A4, and CYP7A1)	(47)
3 iPSC cell lines	Growth factors and cytokine (Activin A, BMP-4, b-FGF, HGF and Oncostatin M)	Entire life cycle reporter virus (Gluc, RFP-NLS-IPS) inhibition with anti-NS5b and Anti-protease	15% of PHH secreted albumin and presence of AFP	Albumin, Alpha-1- anti trypsin, miR-122 and HNF-3beta	(48)
1 iPSC cell line and hESC H9	Growth factors and cytokine (Activin A, Wnt-3, b-FGF, FGF-10, retinoic acid, SB431542, HGF and EGF)	Entire life cycle Inhibition with anti-E2, anti-SRB1 and IFN Patient serum infection (genotype 1a, 1b)	50% of PHH secreted albumin and presence of AFP	Albumin, Alpha-1- anti trypsin	(49)
iPSC cell line and hESC H9	Growth factors and cytokine (Activin A, Wnt-3, BMP-4, a-FGF and HGF)	JFH-1 Entire life cycle Inhibition with anti-NS5b, anti-protease and Cyclophilin inhibors	Presence of AFP	P-450s (CYP3A4/5) Albumin	(54)
1 iPSC cell line	Growth factors and cytokine (Activin A, BMP-4, b-FGF, HGF and Oncostatin M)	HBV life cycle with production of HBsAg and circular DNA	15-20% of PHH secreted albumin Presence of AFP	Albumin Alpha-1- anti trypsin HNF4-Alpha Cytokeratine-18	(37)

5.1. HLCs are permissive to HCV

Most recently, several studies have reported that HLCs differentiated from either ESC or iPSC are prone to support HCV infection (Table 1). Yoshida and colleagues showed that HLCs obtained by differentiating iPSC using both growth factors and an adenovirus expressing SOX17, HEX and HNF4alpha expressed all virus entry receptors and were permissive to pseudoparticles (47). Later, two other studies confirmed that HLCs are permissive to different forms of HCV (viral pseudoparticles and JFH-1; HCVpp and HCVcc) and support the entire viral replication cycle (48,49). Moreover, HLCs are permissive to genotypes 1a, 1b and 2a, support the entire viral cycle and have the ability to generate innate inflammatory responses (TNF-alpha and IL-28B secretion). They are also permissive to genotype 1a and 1b isolated from patient sera (45), which was not possible with hepatoma cell lines. Of note, this model is offering precious information such as the moment when the cells become permissive during the differentiation process and the host factors needed for the infection. Therefore, Wu and collaborators showed that hepatic progenitor cells were persistently infected and that the permissiveness is correlated with the expression of the liver-specific microRNA-122 and cellular factors that affect HCV replication (48). Even if the HLCs support the HCV cycle, the production of viral particles remains very low compared to what has been seen for Huh-7 cell line. By inhibiting the IFN response through the inhibition of JAK/STAT pathway, Zhou and colleagues were able to improve viral infection and replication in HLCs (50). It is well known that adaptation of JFH-1 to cell culture (Huh-7 for example) generates mutations in different regions of viral genome. These mutations responsible for enhancing the virus titter in Huh-7 are either maintained in animal model or reverted to wild type (20). The quantity of viral particles produced when HLCs were used is low and the apparition of mutations has not been investigated yet in this model. Surprisingly, the number of iPSC cell lines that have been differentiated into HLCs used for infection is very low, not exceeding 8 cell lines (Table 1). This observation suggests that either the generation of HLCs from iPSC is more challenging, or the obtained HLCs are less sensitive to HCV infection. Several arguments are in favour of the first hypothesis like, for instance, the reported difference in propensities of iPSC clones to differentiate to HLCs (44). Thus, the improvement of iPSC differentiation protocols and HLCs culture (3D dimension or co-culture with other cell types) conditions must be met if the scientific community wants to provide the field with the ideal model. Accumulating experimental evidences suggest that HLCs are predisposed to differentiate to fully adult hepatocytes when they find themselves in a favourable environment such as a liver bud made by the co-culture of HLCs and endothelial cells (51,52). Furthermore, engraftment of HLCs into mice has contributed to the loss of foetal hepatic markers, such as AFP, and the increase of expression of mature hepatocytes markers like CYP450 isoforms. The engrafted HLCs also become permissive to different genotypes of HCV from chimpanzee sera (53).

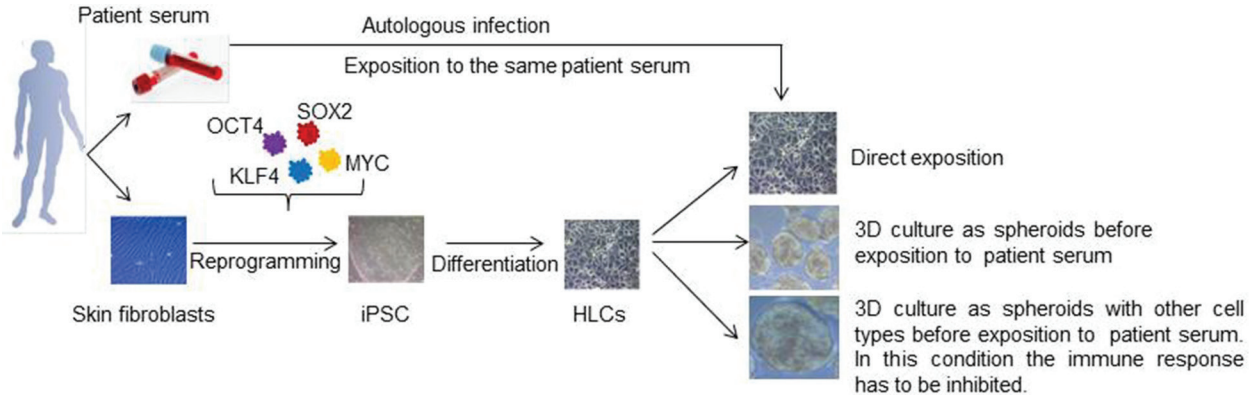

Figure 1. Schematic representation of HCV/HBV autologous infection and other liver pathogens studies and strategies to ameliorate HLCs maturation.

5.2. HBV is able to infect HLCs

During HLCs differentiation, different host factors crucial to HBV infection are expressed sequentially as are many other hepatocytes markers. This finding suggests that these cells are prone to support the HBV life cycle. The first confirmation comes from the work of Shlomai and colleagues, who reported that HLCs, originated from iPSC differentiation, are permissive to HBV infection. This is characterized by mRNA expression, HBsAg secretion and cccDNA accumulation. The exposed HLCs to HBV have been shown to induce their IFN-stimulated genes at a lower level compared to PHH (37). To date the work of Shlomai and colleagues is still unique; it is difficult however to conclude if the HBV physiopathology in iPSC derived hepatocytes will face the same challenges as HCV. Thus, a large number of experiments involving HLCs issued from different iPSC clones is needed.

6. CONCLUSION

For future studies, the generation of HLCs from reprogrammed somatic cells from patients with HCV or HBV infection seems to be crucial to investigate the autologous infection. This direct infection by the same patient serum is important for studying the mechanisms of virus-host cell adaptation. One may speculate that iPSC generated from patients with a known genetic background may impact infection and serve as a model to study the influence of innate immunity on the infection which should significantly push forward our knowledge on antiviral therapy. Another attractive approach to study the virus cycles in these cells is the use of genetically modified iPSC to generate HLCs with the desired phenotypes. This approach may permit a better targeting and understanding of the implications of various host factors in the entire life cycle. To date, many hepatotropic viruses are very difficult to culture *in vitro* probably because of the lack of adequate cell culture models that recapitulate the liver environment. The progress undertaken by different laboratories to culture HLCs in an architecture resembling that of the liver may allow the "culturomics" of these viruses in a personalized manner.

In summary, HLCs are permissive to both HCV and HBV viruses. The exposition of HLCs from a patient (infected with HCV or HBV) to his own serum will generate data crucial to understand host/pathogen interactions (Figure 1). Even with the aforementioned limitations, the role that these cells may play in the field of hepatic disease modelling is unlimited. While the research on HCV may slow down because of the success of the new therapies, other hepatotropic pathogens (hepatitis A, E and D viruses) are in need for such model to uncover their physiology at the molecular level and find new efficient antiviral therapy.

7. ACKNOWLGEMENTS

We would like to thank Professor Sandrine Castelain from CHU-Picardie Amiens France, and Shawn D. Keil from TERUMO-BCT Denver CO. USA, for their critical review of the manuscript. This work was supported by the Region of Picardie.

8. REFERENCES

1. AA Evans, WT London, RG Gish, C Cohen, TM Block. Chronic HBV infection outside treatment guidelines: is treatment needed? *Antivir Ther* 18:229-235 (2013) DOI: 10.3851/IMP2325
2. JD Yang, LR Roberts. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 7:448-458 (2010) DOI: 10.1038/nrgastro.2010.100
3. WHO. HepC WHO fact sheet 164. http://www.hcvka.de/pdf/HepC%20WHO%20fact%20sheet%20164.pdf. (2011)

4. P Sun, X Zhou, SL Farnworth, AH Patel, DC Hay. Modeling human liver biology using stem cell-derived hepatocytes. *Int J Mol Sci* 14:22011-22021 (2013)
DOI: 10.3390/ijms141122011

5. K Takahashi, S Yamanaka. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663-676 (2006)
DOI: 10.1016/j.cell.2006.07.024

6. J Li, W Song, G Pan, J Zhou. Advances in understanding the cell types and approaches used for generating induced pluripotent stem cells. *J Hematol Oncol* 7:50 (2014)
DOI: 10.1186/s13045-014-0050-z

7. J Cai, Y Zhao, Y Liu, F Ye, Z Song, H Qin, S Meng, Y Chen, R Zhou, X Song, Y Guo, M Ding, H Deng. Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatology* 45:1229-1239 (2007)
DOI: 10.1002/hep.21582

8. Y Duan, A Catana, Y Meng, N Yamamoto, S He, S Gupta, SS Gambhir, MA Zern. Differentiation and enrichment of hepatocyte-like cells from human embryonic stem cells in vitro and in vivo. *Stem Cells* 25:3058-3068 (2007)
DOI: 10.1634/stemcells.2007-0291

9. DC Hay, J Fletcher, C Payne, JD Terrace, RC Gallagher, J Snoeys, JR Black, D Wojtacha, K Samuel, Z Hannoun, A Pryde, C Filippi, IS Currie, SJ Forbes, JA Ross, PN Newsome, JP Iredale. Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. *Proc Natl Acad Sci U S A* 105:12301-12306 (2008)
DOI: 10.1073/pnas.0806522105

10. ST Rashid, S Corbineau, N Hannan, SJ Marciniak, E Miranda, G Alexander, I Huang-Doran, J Griffin, L Ahrlund-Richter, J Skepper, R Semple, A Weber, DA Lomas, L Vallier. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J Clin Invest* 120:3127-3136 (2010)
DOI: 10.1172/JCI43122

11. K Si-Tayeb, FK Noto, M Nagaoka, J Li, MA Battle, C Duris, PE North, S Dalton, SA Duncan. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology* 51:297-305 (2010)
DOI: 10.1002/hep.23354

12. RL Gieseck 3rd, NR Hannan, R Bort, NA Hanley, RA Drake, GW Cameron, TA Wynn, L Vallier. Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture. *PLoS One* 9:e86372 (2014)
DOI: 10.1371/journal.pone.0086372

13. V Lohmann, F Korner, J Koch, U Herian, L Theilmann, R Bartenschlager. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 285:110-113 (1999)
DOI: 10.1126/science.285.5424.110

14. B Bartosch, J Dubuisson, FL Cosset. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. *J Exp Med* 197:633-642 (2003)
DOI: 10.1084/jem.20021756

15. T Wakita, T Pietschmann, T Kato, T Date, M Miyamoto, Z Zhao, K Murthy, A Habermann, HG Krausslich, M Mizokami, R Bartenschlager, TJ Liang. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 11:791-796 (2005)
DOI: 10.1038/nm1268

16. J Zhong, P Gastaminza, G Cheng, S Kapadia, T Kato, DR Burton, SF Wieland, SL Uprichard, T Wakita, FV Chisari. Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci U S A* 102:9294-9299 (2005)
DOI: 10.1073/pnas.0503596102

17. BD Lindenbach, MJ Evans, AJ Syder, B Wolk, TL Tellinghuisen, CC Liu, T Maruyama, RO Hynes, DR Burton, JA McKeating, CM Rice. Complete replication of hepatitis C virus in cell culture. *Science* 309:623-626 (2005)
DOI: 10.1126/science.1114016

18. JM Berke, D Moradpour. Hepatitis C virus comes full circle: production of recombinant infectious virus in tissue culture. *Hepatology* 42:1264-1269 (2005)
DOI: 10.1002/hep.20980

19. T Pietschmann, A Kaul, G Koutsoudakis, A Shavinskaya, S Kallis, E Steinmann, K Abid, F Negro, M Dreux, FL Cosset, R Bartenschlager. Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. *Proc Natl Acad Sci U S A* 103:7408-7413 (2006)
DOI: 10.1073/pnas.0504877103

20. A Kaul, I Woerz, P Meuleman, G

Leroux-Roels, R Bartenschlager. Cell culture adaptation of hepatitis C virus and in vivo viability of an adapted variant. *J Virol* 81:13168-13179 (2007)
DOI: 10.1128/JVI.01362-07

21. T Pietschmann, M Zayas, P Meuleman, G Long, N Appel, G Koutsoudakis, S Kallis, G Leroux-Roels, V Lohmann, R Bartenschlager. Production of infectious genotype 1b virus particles in cell culture and impairment by replication enhancing mutations. *PLoS Pathog 5*:e1000475 (2009)
DOI: 10.1371/journal.ppat.1000475

22. E Steinmann, T Pietschmann. Cell culture systems for hepatitis C virus. *Curr Top Microbiol Immunol* 369:17-48 (2013)
DOI: 10.1007/978-3-642-27340-7_2

23. R Bartenschlager, T Pietschmann. Efficient hepatitis C virus cell culture system: what a difference the host cell makes. *Proc Natl Acad Sci U S A* 102:9739-9740 (2005)
DOI: 10.1073/pnas.0504296102

24. K Melen, P Keskinen, A Lehtonen, I Julkunen. Interferon-induced gene expression and signaling in human hepatoma cell lines. *J Hepatol* 33:764-772 (2000)
DOI: 10.1016/S0168-8278(00)80308-6

25. K Banaudha, JM Orenstein, T Korolnek, GC St Laurent 3rd, T Wakita, A Kumar. Primary hepatocyte culture supports hepatitis C virus replication: a model for infection-associated hepatocarcinogenesis. *Hepatology* 51:1922-1932 (2010)
DOI: 10.1002/hep.23616

26. C Gondeau, P Briolotti, F Razafy, C Duret, PA Rubbo, F Helle, T Reme, MP Ripault, J Ducos, JM Fabre, J Ramos, EI Pecheur, D Larrey, P Maurel, M Daujat-Chavanieu. In vitro infection of primary human hepatocytes by HCV-positive sera: insights on a highly relevant model. *Gut* (2013)
DOI: 10.1136/gutjnl-2013-304623

27. J Dias, M Gumenyuk, H Kang, M Vodyanik, J Yu, JA Thomson, II Slukvin. Generation of red blood cells from human induced pluripotent stem cells. *Stem Cells Dev* 20:1639-1647 (2011)
DOI: 10.1089/scd.2011.0078

28. C Terry, RD Hughes, RR Mitry, SC Lehec, A Dhawan. Cryopreservation-induced nonattachment of human hepatocytes: role of adhesion molecules. *Cell Transplant* 16:639-647 (2007)
DOI: 10.3727/000000007783465000

29. S Marukian, L Andrus, TP Sheahan, CT Jones, ED Charles, A Ploss, CM Rice, LB Dustin. Hepatitis C virus induces interferon-lambda and interferon-stimulated genes in primary liver cultures. *Hepatology* 54:1913-1923 (2011)
DOI: 10.1002/hep.24580

30. O Hantz, R Parent, D Durantel, P Gripon, C Guguen-Guillouzo, F Zoulim. Persistence of the hepatitis B virus covalently closed circular DNA in HepaRG human hepatocyte-like cells. *J Gen Virol* 90:127-135 (2009)
DOI: 10.1099/vir.0.004861-0

31. H Yan, G Zhong, G Xu, W He, Z Jing, Z Gao, Y Huang, Y Qi, B Peng, H Wang, L Fu, M Song, P Chen, W Gao, B Ren, Y Sun, T Cai, X Feng, J Sui, W Li. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife 3 (*2014)
DOI: 10.7554/elife.05570

32. SL Christian, TW Collier, D Zu, M Licursi, CM Hough, K Hirasawa. Activated Ras/MEK inhibits the antiviral response of alpha interferon by reducing STAT2 levels. *J Virol* 83:6717-6726 (2009)
DOI: 10.1128/JVI.02213-08

33. Y Tsukada, K Miyazawa, N Kitamura. High intensity ERK signal mediates hepatocyte growth factor-induced proliferation inhibition of the human hepatocellular carcinoma cell line HepG2. *J Biol Chem* 276:40968-40976 (2001)
DOI: 10.1074/jbc.M010890200

34. D Yang, C Zuo, X Wang, X Meng, B Xue, N Liu, R Yu, Y Qin, Y Gao, Q Wang, J Hu, L Wang, Z Zhou, B Liu, D Tan, Y Guan, H Zhu. Complete replication of hepatitis B virus and hepatitis C virus in a newly developed hepatoma cell line. *Proc Natl Acad Sci U S A* 111:E1264-1273 (2014)
DOI: 10.1073/pnas.1320071111

35. P Gripon, C Diot, N Theze, I Fourel, O Loreal, C Brechot, C Guguen-Guillouzo. Hepatitis B virus infection of adult human hepatocytes cultured in the presence of dimethyl sulfoxide. *J Virol* 62:4136-4143 (1988)
DOI Not Found

36. P Gripon, C Diot, C Guguen-Guillouzo. Reproducible high level infection of cultured adult human hepatocytes by hepatitis B virus: effect of polyethylene glycol on adsorption and penetration. *Virology* 192:534-540 (1993) DOI: 10.1006/viro.1993.1069

37. A Shlomai, RE Schwartz, V Ramanan, A Bhatta, YP de Jong, SN Bhatia, CM Rice. Modeling host interactions with hepatitis B virus using primary and induced pluripotent stem cell-derived hepatocellular systems. *Proc Natl Acad Sci U S A* 111:12193-12198 (2014) DOI: 10.1073/pnas.1412631111

38. D Kremsdorf, N Brezillon. New animal models for hepatitis C viral infection and pathogenesis studies. *World J Gastroenterol* 13:2427-2435 (2007) DOI: 10.3748/wjg.v13.i17.2427

39. E Billerbeck, Y de Jong, M Dorner, C de la Fuente, A Ploss. Animal models for hepatitis C. *Curr Top Microbiol Immunol* 369:49-86 (2013) DOI: 10.1007/978-3-642-27340-7_3

40. JL Heckel, EP Sandgren, JL Degen, RD Palmiter, RL Brinster. Neonatal bleeding in transgenic mice expressing urokinase-type plasminogen activator. *Cell* 62:447-456 (1990) DOI: 10.1016/0092-8674(90)90010-C

41. M Grompe, M al-Dhalimy, M Finegold, CN Ou, T Burlingame, NG Kennaway, P Soriano. Loss of fumarylacetoacetate hydrolase is responsible for the neonatal hepatic dysfunction phenotype of lethal albino mice. *Genes Dev* 7:2298-2307 (1993) DOI: 10.1101/gad.7.12a.2298

42. L Sandmann, A Ploss. Barriers of hepatitis C virus interspecies transmission. *Virology* 435:70-80 (2013) DOI: 10.1016/j.virol.2012.09.044

43. Z Song, J Cai, Y Liu, D Zhao, J Yong, S Duo, X Song, Y Guo, Y Zhao, H Qin, X Yin, C Wu, J Che, S Lu, M Ding, H Deng. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. *Cell Res* 19:1233-1242 (2009) DOI: 10.1038/cr.2009.107

44. T Touboul, NR Hannan, S Corbineau, A Martinez, C Martinet, S Branchereau, S Mainot, H Strick-Marchand, R Pedersen, J Di Santo, A Weber, L Vallier. Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. *Hepatology* 51:1754-1765 (2010) DOI: 10.1002/hep.23506

45. J Ge D, Fellay, AJ Thompson, JS Simon, KV Shianna, TJ Urban, EL Heinzen, P Qiu, AH Bertelsen, AJ Muir, M Sulkowski, JG McHutchison, DB Goldstein. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 461:399-401 (2009) DOI: 10.1038/nature08309

46. MA Feitelson, RH Miller. X gene-related sequences in the core gene of duck and heron hepatitis B viruses. *Proc Natl Acad Sci U S A* 85:6162-6166 (1988) DOI: 10.1073/pnas.85.16.6162

47. T Yoshida, K Takayama, M Kondoh, F Sakurai, H Tani, N Sakamoto, Y Matsuura, H Mizuguchi, K Yagi. Use of human hepatocyte-like cells derived from induced pluripotent stem cells as a model for hepatocytes in hepatitis C virus infection. *Biochem Biophys Res Commun* 416:119-124 (2011) DOI: 10.1016/j.bbrc.2011.11.007

48. RE Schwartz, K Trehan, L Andrus, TP Sheahan A, Ploss, SA Duncan, CM Rice, SN Bhatia. Modeling hepatitis C virus infection using human induced pluripotent stem cells. *Proc Natl Acad Sci U S A* 109:2544-2548 (2012) DOI: 10.1073/pnas.1121400109

49. X Wu, JM Robotham, E Lee, S Dalton, NM Kneteman, DM Gilbert, H Tang. Productive hepatitis C virus infection of stem cell-derived hepatocytes reveals a critical transition to viral permissiveness during differentiation. *PLoS Pathog 8*:e1002617 (2012) DOI: 10.1371/journal.ppat.1002617

50. X Zhou, P Sun, B Lucendo-Villarin, AG Angus, D Szkolnicka, K Cameron, SL Farnworth, AH Patel, DC Hay. Modulating innate immunity improves hepatitis C virus infection and replication in stem cell-derived hepatocytes. *Stem Cell Reports* 3:204-214 (2014) DOI: 10.1016/j.stemcr.2014.04.018

51. T Takebe, K Sekine, M Enomura, H Koike, M Kimura, T Ogaeri, RR Zhang, Y Ueno, YW Zheng, N Koike, S Aoyama, Y Adachi, H Taniguchi. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499:481-484 (2013) DOI: 10.1038/nature12271

52. T Takebe, RR Zhang, H Koike, M Kimura, E Yoshizawa, M Enomura, N Koike, K Sekine, H Taniguchi. Generation of a vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nat Protoc* 9:396-409 (2014)
DOI: 10.1038/nprot.2014.020

53. A Carpentier, A Tesfaye, V Chu, I Nimgaonkar, F Zhang, SB Lee, SS Thorgeirsson, SM Feinstone, TJ Liang. Engrafted human stem cell-derived hepatocytes establish an infectious HCV murine model. *J Clin Invest* 124:4953-4964 (2014)
DOI: 10.1172/JCI75456

54. P Roelandt, S Obeid, J Paeshuyse, J Vanhove, A Van Lommel, Y Nahmias, F Nevens, J Neyts, CM Verfaillie. Human pluripotent stem cell-derived hepatocytes support complete replication of hepatitis C virus. *J Hepatol*. 57:246-251 (2012)
DOI: 10.1016/j.jhep.2012.03.030

Key Words: iPSC, Hepatocyte- like cells, HBV, HCV, Differentiation, Review

Send correspondence to: Abderrahmane Bengrine; Biobanque de Picardie CHU Amiens-Picardie 80054 Amiens cedex 1, France. Tel: 0033322089360, Fax: 0033322089365, E-mail: abengrine@biobanque-picardie.com