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REVIEW

Humanized Murine Model for HBV and HCV Using Human Induced Pluripotent Stem Cells

Xiao-Ling Zhou[1,2], Gareth J. Sullivan[3,4], Pingnan Sun[1,5], and In-Hyun Park[1]

[1]Department of Genetics, Yale Stem Cell Center, Yale School of Medicine, New Haven. CT. 06520, USA, [2]Department of Cell Biology and Genetics, Shantou University Medical College, China, [3]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, EH16 4SB, UK, [4]Stem Cell Epigenetics Laboratory, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, and Norwegian Center for Stem Cell Research, 0317 Oslo, Norway, and [5]Department of Pathology, Shantou University Medical College, China

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Infection of hepatitis B virus (HBV) and hepatitis C virus (HCV) results in heterogeneous outcomes from acute asymptomatic infection to chronic infection leading to cirrhosis and hepatocellular carcinoma (HCC). *In vitro* models using animal hepatocytes, human HCC cell lines, or *in vivo* transgenic mouse models have contributed invaluably to understanding the pathogenesis of HBV and HCV. A humanized mouse model made by reconstitution of human primary hepatocytes in the liver of the immunodeficient mouse provides a novel experimental opportunity which mimics the *in vivo* growth of the human hepatocytes. The limited access to primary human hepatocytes necessitated the search for other cellular sources, such as pluripotent stem cells. Human embryonic stem cells (hESCs) have the features of self-renewal and pluripotency and differentiate into cells of all three germ layers, including hepatocytes. Human-induced pluripotent stem cells (iPSCs) derived from the patient's or individual's own cells provide a novel opportunity to generate hepatocyte-like cells with the defined genetic composition. Here, we will review the current perspective of the models used for HBV and HCV study, and introduce the personalized mouse model using human iPSCs. This novel mouse model will facilitate the direct investigation of HBV and HCV in human hepatocytes as well as probing the genetic influence on the susceptibility of hepatocytes to HBV and HCV.

Key words: HBV, HCV, Humanized model, iPSCs, Reprogramming

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses that can cause acute and chronic diseases in liver after infection. It is estimated that more than 2 billion people have been infected with HBV and the annual death toll due to HBV infection is more than 600,000 (Ganem and Prince, 2004). The global HCV infection was estimated to be 3% or 170 million individuals, and more than 350,000 fatalities are due to HCV-related liver disease each year (Te and Jensen, 2010; Yang and Roberts, 2010). People chronically infected with HBV or HCV have a higher risk of liver cirrhosis or hepatocellular carcinoma (HCC). According to a WHO report, 90% of infants infected with HBV during their first year of life develop chronic infection. 30 to 50% of children infected between the ages of one to four years develop a chronic condition, while about 90% of healthy adults infected with HBV will recover and be completely devoid of the virus within six months. 10 to 30% of the individual infected with the acute HCV recover, while the rest may develop into chronic carriers after the acute stage (Afdhal, 2004). Chronic HBV and HCV infection has huge economic burdens in countries with endemic infection (Yang and Roberts, 2010).

The distribution of HBV or HCV occurrence is varied geographically. The endemic levels are within 8 to

Correspondence to: In-Hyun Park, Department of Genetics, Yale Stem Cell Center, Yale School of Medicine, 10 Amistad, 201B, New Haven. CT. 06520, USA
Tel: 1-203-737-4189, Fax: 1-203-785-7095
E-mail: inhyun.park@yale.edu

15% in many areas including China, South-East Asia, most of Africa, most Pacific Islands, parts of the Middle East and the Amazon Basin. Meanwhile less than 2% of the population are infected with HBV or HCV in the United States, Western Europe and Australia (Te and Jensen, 2010). There are not only cultural differences among these countries, but also differences in the population-level genetic background that have been proposed to be responsible for the difference. Upon infection, individuals present a spectrum of symptoms from an acute symptomatic illness to chronic conditions, which lead to cirrhosis and HCC. According to the epidemiological study, there are individual and regional differences in terms of the susceptibility to acquire HBV or HCV. Hepatitis B vaccine has been available since 1982 and is 95% effective in preventing infection. However, most chronic carriers of HBV are threatened by the development of cirrhosis and HCC. At present, there are no effective Hepatitis C vaccines available, partially due to the rapid mutation rate of the HCV genome and the limited knowledge of HCV pathogenesis. Understanding the detailed mechanism of entry into and propagation in cells is imperative for the development of effective vaccines and antivirals for HCV (Washburn et al., 2011).

Research on HBV or HCV has been hampered by the difficulty in culturing human primary hepatocytes. They tend to differentiate and lose hepatic function after a limited period of *in vitro* culture. Thus, alternative models have been used. Animal hepatocytes, HCC cell lines, or transgenic mouse models have contributed to understanding the pathogenesis of HBV and HCV. Despite the success, there are shortcomings in those models, such that they do not properly model *in vivo* human hepatocytes. Other alternative cellular sources have been sought to make a model closer to the human primary hepatocytes. Human embryonic stem cells (hESCs) have the capacity of self-renewal and pluripotency (Murry and Keller, 2008). The pluripotency allows for generation of theoretically all cell types in the body, including hepatocytes. The indefinite self-renewing feature of hESCs promises the continuous supply of hepatocyte with the same genetic composition. The recent development of human induced pluripotent stem cells (iPSCs) even provides cells of the defined genetic background from any patients or individuals (Hanna et al., 2010). In this review, we will give an overview of the model systems used in studying the HBV and HCV and will discuss the novel model based on the human pluripotent stem cells.

MODELS TO INVESTIGATE HBV OR HCV PATHOGENESIS

Models using cell lines or animals have been developed for *in vitro* and *in vivo* investigation of HBV or HCV (Tables I and II). Despite the limitations, each model contributes to understanding the fundamentals of HBV and HCV pathogenesis and to the development of vaccines for HBV. The duck HBV (DHBV) primary hepatocyte model aided the discovery of key features of HBV such as virus structure, genome and mechanisms of replication (Yokosuka et al., 1988; Seigneres et al., 2001). Furthermore, this model facilitated the development of the first oral antiviral drug for HBV – lamivudine (Lee et al., 1989; Fischer and Tyrrell, 1996; Tomita et al., 2000). However, the DHBV model has shortcomings in modeling human HBV, because DHBV does not express Protein X found in human hepadnaviruses, which is presumed to be critical for the development of HCC by human HBV (Feitelson and Miller, 1988).

Models biologically more relevant to the human system have been established using HCC cell lines including HepG2, Chang, Hep3B and Huh7. These cell lines have been useful *in vitro* models for production of the infective HBV virions (Sells et al., 1988) and drug screening (Sun and Nassal, 2006). However, there are limitations with these models as well. They are refractory to HBV infection due to the loss of features of primary human hepatocytes, such as the expression of the specific receptors for HBV (Glebe and Urban, 2007). Therefore, these cell lines are not the optimal models for investigating early steps of human HBV infection (Garcia et al., 2002; Mee et al., 2009). In addition, these cell lines are derived from HCC that had already become malignant and may not be a suitable model to study the progressive development of HCC caused by either HBV or HCV.

Animal models based on the expression of HBV in the transgenic mouse have been useful for investigating HBV pathogenesis and for developing antiviral drugs. However, HBV replication is minimal in HBV transgenic mice (Araki et al., 1989). They also present an acute phenotype rather than the chronic disease due to the transgene tolerance (Moriyama et al., 1990). By providing syngeneic unprimed splenocytes, scientists have developed an improved transgenic model for the chronic HBV in immunodeficient mice (Larkin et al., 1999).

Primary human hepatocytes may represent the model biologically most relevant in investigating HBV or HCV pathogenesis. However, human primary hepatocytes are genetically diverse and show a large

Table I. HBV *in vitro* and *in vivo* models

HBV Model	Features	Shortcomings	Important Findings/ highlights	References
Primary human hepatocytes	Closest to natural status for virus infection; Good system for searching real HBV receptors	Difficult to obtain and maintain; lose the function of human primary hepatocytes soon	Suitable for elucidating the entry process of HBV; test anti-HBV drug *in vitro*	(Ochiya et al., 1989; Ren and Nassal, 2001; Schulze-Bergkamen et al., 2003)
Human hepatoma cell lines	Easy to culture *in vitro* and obtain the stable results	Lack the characteristics of primary humanhepatocyte; malignant cells	HepG2.2.15 with full-length HBV is capable of replicating the viral genome and producing progeny Virus; Useful in virion production and drug screening	(Sells et al., 1988; Chouteau et al., 2001; Sun and Nassal, 2006)
Chimpanzee model	Human HBV can infect chimpanzee liver and replicate in it	High expenditure, ethical problems	Served as a useful means to study the mechanism of hepatitis B viral progression to chronic liver disease	(Pancholi et al., 2001; Kamili et al., 2009)
Duck HBV primary hepatocyte culture model	Duck HBV has similar biologicalfeatures of human HBV	Lack of Protein X of human hepadnaviruses	Helpful in investigating virus structure, genome, and mechanisms of replication; accelerated the discovery of first anti-HBV drug	(Feitelson and Miller, 1988; Yokosuka et al., 1988; Fischer and Tyrrell, 1996; Tomita et al., 2000; Seigneres et al., 2001)
Transgenic HBV mouse model	Can investigate HBV *in vivo*	Low virus replication, transgene tolerance	Helpful in chemotherapy and drug screening for HBV	(Kajino et al., 1997; Chemin et al., 1999; Larkin et al., 1999; Weber et al., 2002; Barone et al., 2006; Yu et al., 2011)
Tupaia model	Tupaia are squirrel-sized animals closely related to primates	Low infection rates *in vivo*; does not show a persistent HBV infection *in vitro*	Improved the understanding of the fulminant clinical course associated with HBV mutations	(Walter et al., 1996; Glebe et al., 2003; Baumert et al., 2005; Glebe et al., 2005)
A chimeric mouse model with human hepatocytes	Maintain the pristine human-hepatocyte-like features	Low repopulation ratio, limited resource of human hepatocytes; lack of an immune system	A useful tool for the study of HBV virology and evaluation of anti-HBV drugs	(Tsuge et al., 2005; Sugiyama et al., 2007; Tabuchi et al., 2008; Tanaka et al., 2008; Robinet and Baumert, 2010; Lutgehetmann et al., 2011)

variation among isolates. In addition, obtaining and maintaining them for a long time in *in vitro* cultures is challenging. For example, they tend to lose the susceptibility to HBV infection after culturing *in vitro*. Thus, there have been many attempts to reconstitute mouse liver with human primary hepatocytes to maintain the pristine hepatocyte-like features. In order to give growth advantage to donor human hepatocytes, urokinase-typeplasminogen activator (uPA) transgenic or fumarylacetoacetate hydrolase (Fah)-deficient mouse models have been developed. Albumin-promoter driven expression of uPA is toxic to the hepatocytes, which undergo continual necrosis, leading to an insensitive stimulus for liver regeneration (Sandgren et al., 1991). Likewise, the absence of the tyrosine catabolic enzyme, fumarylacetoaetate hydrolase (Fah), causes liver failure in Fah-/- mouse due to the accumulation of toxic compounds in blood and urine. Feeding Fah-/-mice with NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexnedione) prevents the liver failure (Dandri et al., 2001; Mercer et al., 2001; Bissig et al., 2007). By crossing the Fah-/- mouse with immunodeficient Rag2-/- (recombination activating gene 2) and IL2rg-/- (IL-2 receptor common γ-chain) mice, Bissig et al. have generated a Fah-/-Rag2-/-IL2rg-/-mouse, which lacks mature B and T lymphocytes as well as NK cells (Bissig et al., 2007). These triple KO mice make it possible for more efficient engraftment of human hepatocytes.

Table II. HCV *in vitro* and *in vivo* models

HCV Model	Advantages	Shortcomings	Important Findings/ highlights	References
Primary human hepatocytes	Closest model for the natural host cell of HCV	Difficult to obtain and maintain, lose the function of human primary hepatocytes soon	This model allows production of infectious HCV	(Nahmias et al., 2006; Jouan et al., 2010; Podevin et al., 2010)
The established human hepatoma cell lines	Easy to culture *in vitro* and obtain the stable results	Lack the characteristics of primary humanhepatocyte; malignant cells	Identify binding proteins to HCV or elements interact with HCV protein; A breakthrough in HCV research was achievedin 2005 when Huh-7 infected with the cloning of JFH1, a genotype 2a-HCV produce workable titers	(Kalkeri et al., 2001; Garcia et al., 2002; Lindenbach et al., 2005; Mee et al., 2009)
Chimpanzee model	HCV replication was detectable in this surrogate model; The only validated *in vivo* model for testing the infectivity of HCV and studying the natural history of HCV	High cost, ethical problems	Played a critical role inthe discovery of HCV; currently, the immunogenicity and efficacy of vaccine candidates against HCV can be tested only in chimpanzees.	(Lu et al., 2001; Mizukoshi et al., 2002; Youn et al., 2005; Puig et al., 2006)
Transgenic HCV mouse model	Can investigate HCV *in vivo*	Usually use only part of HCV elements to generate this model	Valuable in studying the biology of HCV and evaluating antiviral compounds.	(Renard et al., 2000; Wedemeyer et al., 2001; Perlemuter et al., 2003; Shuai et al., 2008)
Tupaia model	Tupaia are squirrel-sized animals closely related to primates	Low infection rates *in vivo*	A potential practical experimental model for studies of HCV infection.	(Barth et al., 2005; Amako et al., 2010)
A chimeric mouse model with human hepatocytes	Maintain the pristine human-hepatocyte-like features, susceptible to infectionwith native HCV	Low repopulation ratio, limited resource of human hepatocytes; lack of an immune system.	Useful tool for evaluating the effect of anti-HCV drugs such as IFN, protease inhibitors and polymerase inhibitors.	(Mercer et al., 2001; Kneteman et al., 2006; Robinet and Baumert, 2010; Washburn et al., 2011)

PLURIPOTENT STEM CELLS FOR HEPATIC DISEASE MODELING

hESCs are derived from cells of inner cell mass of blastocysts of human embryos. Like inner cell mass cells, they have features of self-renewal and pluripotency. The success of reprogramming human somatic cells to generate induced pluripotent stem cells (iPSCs) represents a novel approach for generating patient-specific pluripotent stem cells. Overexpression of four transcription factors (Oct4, octamer-binding transcription factor 4; Sox2, sex determining region Y-box 2; Klf4, Kruppel-like factor 4 and Myc, cellular homologue of avian myelocytomatosis virus oncogene) epigenetically reprograms somatic cells to acquire the pluripotent features. A more detailed description of the reprogramming can be found in recent reviews (Takahashi et al., 2007; Yu et al., 2007; Zwi et al., 2009). iPSCs have the similar features as hESCs and demonstrate self-renewing and pluripotent capacity (Nizzardo et al., 2010; Si-Tayeb et al., 2010; Sullivan et al., 2010). Reprogramming avoids the ethical issues attributed to hESCs. Additionally, iPSCs can be derived from a wide range of individuals with different ethnicities and disease states, facilitating the development of personalized models of disease and autologous regenerative medicine. Human iPSCs are readily used to model genetic and non-genetic diseases, especially for those that lack patient tissue or suitable disease models. iPSCs for a series of neuronal, hematopoeitic and complex diseases have been generated by multiple groups (Park et al., 2008; Marchetto et al., 2010; Zhang et al., 2010). *In vitro* neuronal disease models using iPSCs have been well explored, such as familial dysautonomia, Rett syndrome and spinal muscular dystrophy (Lee et al., 2009; Marchetto et al., 2010; Chang et al., 2011).

Many efforts were made to generate cells of different lineages, including hepatocytes. Initially, sodium butyrate was used to initiate hepatic programming in hESCs differentiated as embryoid bodies (EBs; Rambhatla et al., 2003). Subsequent studies used growth factors that are critical to endodermal tissue

development. The use of Activin A and Wnt3a showed an increase in efficiency of generating hepatocyte-like cells (HLCs) in differentiating cells (Hay et al., 2008). Use of BMP4, which facilitates the formation of meso-endodermal cells, also showed an increase in HLC formation (Gouon-Evans et al., 2006; Zhao et al., 2009). Validation of the function of *in vitro* derived hepatocyte-like cells has been limited. Expression of hepatic markers, such as albumin, AFP and HNFs in *in vitro* differentiated cells has been used to show hepatic differentiation *in vitro*. Faithful functional recovery in liver from mice transplanted with *in vitro* derived hepatocytes or HLCs should be true validation of function (Duncan et al., 2009). Like in primary human hepatocytes, uPA transgenetic mice were used for the successful engraftment of *in vitro* derived hepatocytes (Agarwal et al., 2008).

Human iPSCs have been tested for their potency of differentiating into cells of different lineages, including the endodermal hepatic lineage. We are among the first groups that reported the successful differentiation of human HLCs from normal iPSCs as well as iPSCs from a diabetic patient (Sullivan et al., 2010). Si-Tayeb et al. also described the generation of HLCs from human iPSCs by using hypoxic conditions which produced over 80% HLCs (Si-Tayeb et al., 2010). Recently, the iPSCs from patients with an alpha-1-antitrypsin mutation were generated (Rashid et al., 2010). Alpha-1-antitrypsin (A1AT), a serine protease inhibitor secreted primarily from hepatocytes, irreversibly inhibits neutrophil elastase, cathepsin G and proteinase 3. Mutations in glutamate 342 to lysine causes the accumulation of insoluble aggregations in the endoplasmic reticulum of hepatocytes, leading to autophagy, oxidative stress and apoptosis (Gooptu and Lomas, 2009). iPSCs from patients with A1AT mutations mimic the patient's phenotypes and will provide a platform to drug discovery.

A HUMANIZED MOUSE MODEL FOR HBV OR HCV

The application of personalized human iPSCs has a number of advantages with respect to development of

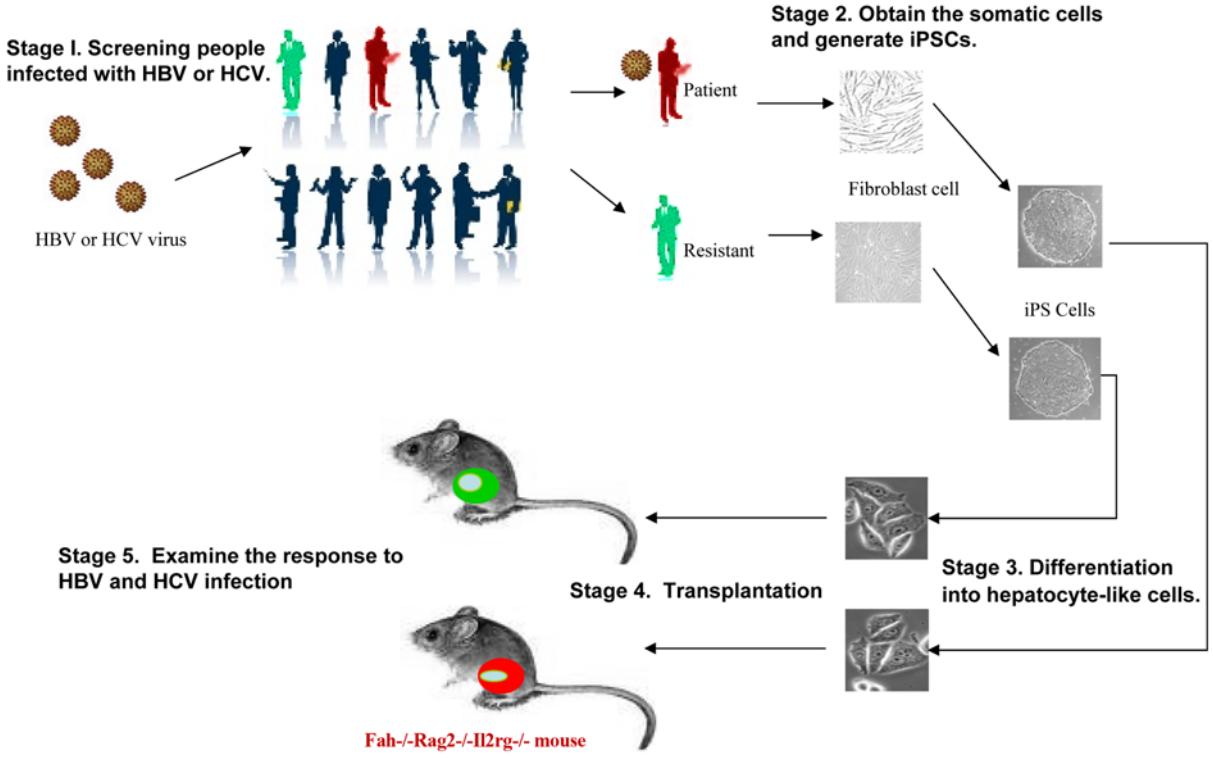

Fig. 1. Strategies for developing a personalized mouse model for HBV and HCV using personalized human iPS cells. A preclinical humanized mouse model can be generated in a step-wide manner. In stage 1, among the individuals who have a history of HBV or HCV infection, those with the chronic infection and those who recovered after asymptomatic infection can be determined. In stage 2, somatic cells from those patients can be used to produce iPSCs, which further differentiate into hepatocyte-like cells *in vitro* in stage 3. The *in vitro* differentiated hepatocyte-like cells can be transplanted to the appropriate immunodeficient recipient mice, such as Fah-/-Rag2-/-Il2rg-/- mice in stage 4. Finally, mice will be infected with HBV or HCV and examined for a response in hepatocytes from susceptible individuals, or resistant individuals (stage 5).

in vitro and *in vivo* models for HBV or HCV research. As discussed in the previous section, human hepatocytes show diverse responsiveness to HBV or HCV, depending on the genetic composition of the individual. Differences in the expression of human leukocyte antigen (HLA) loci, tumor necrosis factor alpha (TNF-α) and mannose binding protein among individuals has been associated with the propensity for the development chronic HBV infection (Thursz et al., 2011).

Because iPSCs can be derived from any individual, their response to HBV and HCV, or even to the drugs can be monitored in iPSC-derived hepatocytes. These patient specific iPSC-derived HLCs can be used to reconstitute the Fah-/-Rag2-/-Il2rg-/- mouse livers that will support the efficient engraftment of human hepatocytes. This humanized mouse liver model can be used to investigate the pathogenesis of HBV and HCV at a personalized level in addition to identifying new therapeutic targets (Fig. 1). Comparative analysis between two groups of people who show differences in susceptibility to HBV or HCV can be directly performed using the *in vivo* reconstituted cells.

CONCLUSION

Here, we have discussed the advantages and shortcomings of current models in HBV and HCV research and a novel chimera model using human iPSCs from people with different reactions to HBV or HCV infection. The success of the humanized mouse model of HBV and HCV would still need further improvement in areas, including the optimization of hepatic differentiation protocols and engraftment of iPSC-derived hepatocytes. Despite these needs, this model would provide a preclinical humanized mouse model for HBV and HCV investigation. The response of two different groups of hepatocytes to HBV or HCV in this mouse model will provide important information about the progressive change in these hepatocytes. In addition, the effect of known or new drugs for HBV or HCV can be tested for their efficacy in this model before being tested in patients. This novel personalized hepatitis mouse model will provide the opportunity to clarify the still unclear pathogenic mechanisms of HBV or HCV infection and to develop a platform for antivirus drug screening.

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REFERENCES

Afdhal, N. H., The natural history of hepatitis C. *Semin. Liver Dis.*, 24 Suppl 2, 3-8 (2004).

Agarwal, S., Holton, K. L., and Lanza, R., Efficient differentiation of functional hepatocytes from human embryonic stem cells. *Stem Cells,* 26, 1117-1127 (2008).

Amako, Y., Tsukiyama-Kohara, K., Katsume, A., Hirata, Y., Sekiguchi, S., Tobita, Y., Hayashi, Y., Hishima, T., Funata, N., Yonekawa, H., and Kohara, M., Pathogenesis of hepatitis C virus infection in *Tupaia belangeri*. *J. Virol.*, 84, 303-311 (2010).

Araki, K., Miyazaki, J., Hino, O., Tomita, N., Chisaka, O., Matsubara, K., and Yamamura, K., Expression and replication of hepatitis B virus genome in transgenic mice. *Proc. Natl. Acad Sci. U. S. A.,* 86, 207-211 (1989).

Barone, M., Spano, D., D'Apolito, M., Centra, M., Lasalandra, C., Capasso, M., Di Leo, A., Volinia, S., Arcelli, D., Rosso, N., Francavilla, A., Tiribelli, C., and Iolascon, A., Gene expression analysis in HBV transgenic mouse liver: a model to study early events related to hepatocarcinogenesis. *Mol. Med.*, 12, 115-123 (2006).

Barth, H., Cerino, R., Arcuri, M., Hoffmann, M., Schurmann, P., Adah, M. I., Gissler, B., Zhao, X., Ghisetti, V., Lavezzo, B., Blum, H. E., von Weizsacker, F., Vitelli, A., Scarselli, E., and Baumert, T. F., Scavenger receptor class B type I and hepatitis C virus infection of primary tupaia hepatocytes. *J. Virol.,* 79, 5774-5785 (2005).

Baumert, T. F., Yang, C., Schurmann, P., Kock, J., Ziegler, C., Grullich, C., Nassal, M., Liang, T. J., Blum, H. E., and von Weizsacker, F., Hepatitis B virus mutations associated with fulminant hepatitis induce apoptosis in primary Tupaia hepatocytes. *Hepatology*, 41, 247-256 (2005).

Bissig, K. D., Le, T. T., Woods, N. B., and Verma, I. M., Repopulation of adult and neonatal mice with human hepatocytes: a chimeric animal model. *Proc. Natl. Acad Sci. U. S. A.,* 104, 20507-20511 (2007).

Chang, T., Zheng, W., Tsark, W., Bates, S. E., Huang, H., Lin, R. J., and Yee, J. K., Phenotypic rescue of induced pluripotent stem cell-derived motoneurons of a spinal muscular atrophy patient. *Stem Cells*, 29, 2090-2093 (2011).

Chemin, I., Ohgaki, H., Chisari, F. V., and Wild, C. P., Altered expression of hepatic carcinogen metabolizing enzymes with liver injury in HBV transgenic mouse lineages expressing various amounts of hepatitis B surface antigen. *Liver*, 19, 81-87 (1999).

Chouteau, P., Le Seyec, J., Saulier-Le Drean, B., Cannie, I., Brissot, P., Lescoat, G., Guguen-Guillouzo, C., and Gripon, P., Inhibition of hepatitis B virus production associated with high levels of intracellular viral DNA intermediates in iron-depleted HepG2.2.15 cells. *J. Hepatol.,* 34, 108-113

(2001).
Dandri, M., Burda, M. R., Torok, E., Pollok, J. M., Iwanska, A., Sommer, G., Rogiers, X., Rogler, C. E., Gupta, S., Will, H., Greten, H., and Petersen, J., Repopulation of mouse liver with human hepatocytes and *in vivo* infection with hepatitis B virus. *Hepatology,* 33, 981-988 (2001).
Duncan, A. W., Dorrell, C., and Grompe, M., Stem cells and liver regeneration. *Gastroenterology,* 137, 466-481 (2009).
Feitelson, M. A. and Miller, R. H., 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).
Fischer, K. P. and Tyrrell, D. L., Generation of duck hepatitis B virus polymerase mutants through site-directed mutagenesis which demonstrate resistance to lamivudine [(–)-beta-L-2', 3'-dideoxy-3'-thiacytidine] *in vitro*. *Antimicrob. Agents Chemother.,* 40, 1957-1960 (1996).
Ganem, D. and Prince, A. M., Hepatitis B virus infection--natural history and clinical consequences. *N. Engl. J. Med.,* 350, 1118-1129 (2004).
Garcia, J. E., Puentes, A., Suarez, J., Lopez, R., Vera, R., Rodriguez, L. E., Ocampo, M., Curtidor, H., Guzman, F., Urquiza, M., and Patarroyo, M. E., Hepatitis C virus (HCV) E1 and E2 protein regions that specifically bind to HepG2 cells. *J. Hepatol.,* 36, 254-262 (2002).
Glebe, D., Aliakbari, M., Krass, P., Knoop, E. V., Valerius, K. P., and Gerlich, W. H., Pre-s1 antigen-dependent infection of Tupaia hepatocyte cultures with human hepatitis B virus. *J. Virol.,* 77, 9511-9521 (2003).
Glebe, D. and Urban, S., Viral and cellular determinants involved in hepadnaviral entry. *World J. Gastroenterol.,* 13, 22-38 (2007).
Glebe, D., Urban, S., Knoop, E. V., Cag, N., Krass, P., Grun, S., Bulavaite, A., Sasnauskas, K., and Gerlich, W. H., Mapping of the hepatitis B virus attachment site by use of infection-inhibiting preS1 lipopeptides and tupaia hepatocytes. *Gastroenterology,* 129, 234-245 (2005).
Gooptu, B. and Lomas, D. A., Conformational pathology of the serpins: themes, variations, and therapeutic strategies. *Annu. Rev. Biochem.,* 78, 147-176 (2009).
Gouon-Evans, V., Boussemart, L., Gadue, P., Nierhoff, D., Koehler, C. I., Kubo, A., Shafritz, D. A., and Keller, G., BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm. *Nat. Biotechnol.*, 24, 1402-1411 (2006).
Hanna, J. H., Saha, K., and Jaenisch, R., Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues. *Cell*, 143, 508-525 (2010).
Hay, D. C., Fletcher, J., Payne, C., Terrace, J. D., Gallagher, R. C., Snoeys, J., Black, J. R., Wojtacha, D., Samuel, K., Hannoun, Z., Pryde, A., Filippi, C., Currie, I. S., Forbes, S. J., Ross, J. A., Newsome, P. N., and Iredale, J. P., 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).
Jouan, L., Melancon, P., Rodrigue-Gervais, I. G., Raymond, V. A., Selliah, S., Boucher, G., Bilodeau, M., Grandvaux, N., and Lamarre, D., Distinct antiviral signaling pathways in primary human hepatocytes and their differential disruption by HCV NS3 protease. *J. Hepatol.,* 52, 167-175 (2010).
Kajino, K., Kamiya, N., Yuasa, S., Takahara, T., Sakurai, J., Yamamura, K., and Hino, O., Evaluation of anti-hepatitis B virus (HBV) drugs using the HBV transgenic mouse: application of the semiquantitative polymerase chain reaction (PCR) for serum HBV DNA to monitor the drug efficacy. *Biochem. Biophys. Res. Commun.*, 241, 43-48 (1997).
Kalkeri, G., Khalap, N., Garry, R. F., Fermin, C. D., and Dash, S., Hepatitis C virus protein expression induces apoptosis in HepG2 cells. *Virology,* 282, 26-37 (2001).
Kamili, S., Sozzi, V., Thompson, G., Campbell, K., Walker, C. M., Locarnini, S., and Krawczynski, K., Efficacy of hepatitis B vaccine against antiviral drug-resistant hepatitis B virus mutants in the chimpanzee model. *Hepatology,* 49, 1483-1491 (2009).
Kneteman, N. M., Weiner, A. J., O'Connell, J., Collett, M., Gao, T., Aukerman, L., Kovelsky, R., Ni, Z. J., Zhu, Q., Hashash, A., Kline, J., Hsi, B., Schiller, D., Douglas, D., Tyrrell, D. L., and Mercer, D. F., Anti-HCV therapies in chimeric scid-Alb/uPA mice parallel outcomes in human clinical application. *Hepatology,* 43, 1346-1353 (2006).
Larkin, J., Clayton, M., Sun, B., Perchonock, C. E., Morgan, J. L., Siracusa, L. D., Michaels, F. H., and Feitelson, M. A., Hepatitis B virus transgenic mouse model of chronic liver disease. *Nat. Med.,* 5, 907-912 (1999).
Lee, B., Luo, W. X., Suzuki, S., Robins, M. J., and Tyrrell, D. L., *In vitro* and *in vivo* comparison of the abilities of purine and pyrimidine 2',3'-dideoxynucleosides to inhibit duck hepadnavirus. *Antimicrob. Agents Chemother.,* 33, 336-339 (1989).
Lee, G., Papapetrou, E. P., Kim, H., Chambers, S. M., Tomishima, M. J., Fasano, C. A., Ganat, Y. M., Menon, J., Shimizu, F., Viale, A., Tabar, V., Sadelain, M., and Studer, L., Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature*, 461, 402-406 (2009).
Lindenbach, B. D., Evans, M. J., Syder, A. J., Wolk, B., Tellinghuisen, T. L., Liu, C. C., Maruyama, T., Hynes, R. O., Burton, D. R., McKeating, J. A., and Rice, C. M., Complete replication of hepatitis C virus in cell culture. *Science*, 309, 623-626 (2005).
Lu, L., Nakano, T., Orito, E., Mizokami, M., and Robertson, B. H., Evaluation of accumulation of hepatitis C virus mutations in a chronically infected chimpanzee: comparison of the core, E1, HVR1, and NS5b regions. *J. Virol.,* 75, 3004-3009 (2001).
Lutgehetmann, M., Bornscheuer, T., Volz, T., Allweiss, L., Bockmann, J. H., Pollok, J. M., Lohse, A. W., Petersen, J., and Dandri, M., Hepatitis B virus limits response of human hepatocytes to interferon-alpha in chimeric mice. *Gastroenterology,* 140, 2074-2083 (2011).
Marchetto, M. C., Carromeu, C., Acab, A., Yu, D., Yeo, G. W.,

Mu, Y., Chen, G., Gage, F. H., and Muotri, A. R., A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell*, 143, 527-539 (2010).

Mee, C. J., Harris, H. J., Farquhar, M. J., Wilson, G., Reynolds, G., Davis, C., van, P. Balfe, I. S. C., and McKeating, J. A., Polarization restricts hepatitis C virus entry into HepG2 hepatoma cells. *J. Virol.,* 83, 6211-6221 (2009).

Mercer, D. F., Schiller, D. E., Elliott, J. F., Douglas, D. N., Hao, C., Rinfret, A., Addison, W. R., Fischer, K. P., Churchill, T. A., Lakey, J. R., Tyrrell, D. L., and Kneteman, N. M., Hepatitis C virus replication in mice with chimeric human livers. *Nat. Med.,* 7, 927-933 (2001).

Mizukoshi, E., Nascimbeni, M., Blaustein, J. B., Mihalik, K., Rice, C. M., Liang, T. J., Feinstone, S. M., and Rehermann, B., Molecular and immunological significance of chimpanzee major histocompatibility complex haplotypes for hepatitis C virus immune response and vaccination studies. *J. Virol.,* 76, 6093-6103 (2002).

Moriyama, T., Guilhot, S., Klopchin, K., Moss, B., Pinkert, C. A., Palmiter, R. D., Brinster, R. L., Kanagawa, O., and Chisari, F. V., Immunobiology and pathogenesis of hepatocellular injury in hepatitis B virus transgenic mice. *Science,* 248, 361-364 (1990).

Murry, C. E. and Keller, G., Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell*, 132, 661-680 (2008).

Nahmias, Y., Casali, M., Barbe, L., Berthiaume, F., and Yarmush, M. L., Liver endothelial cells promote LDL-R expression and the uptake of HCV-like particles in primary rat and human hepatocytes. *Hepatology,* 43, 257-265 (2006).

Nizzardo, M., Simone, C., Falcone, M., Locatelli, F., Riboldi, G., Comi, G. P., and Corti, S., Human motor neuron generation from embryonic stem cells and induced pluripotent stem cells. *Cell Mol. Life Sci.,* 67, 3837-3847 (2010).

Ochiya, T., Tsurimoto, T., Ueda, K., Okubo, K., Shiozawa, M., and Matsubara, K., An *in vitro* system for infection with hepatitis B virus that uses primary human fetal hepatocytes. *Proc. Natl. Acad. Sci. U. S. A.,* 86, 1875-1879 (1989).

Pancholi, P., Lee, D. H., Liu, Q., Tackney, C., Taylor, P., Perkus, M., Andrus, L., Brotman, B., and Prince, A. M., DNA prime/canarypox boost-based immunotherapy of chronic hepatitis B virus infection in a chimpanzee. *Hepatology,* 33, 448-454 (2001).

Park, I.-H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., Lensch, M. W., Cowan, C., Hochedlinger, K., and Daley, G. Q., Disease-specific induced pluripotent stem cells. *Cell*, 134, 877-886 (2008).

Perlemuter, G., Letteron, P., Carnot, F., Zavala, F., Pessayre, D., Nalpas, B., and Brechot, C., Alcohol and hepatitis C virus core protein additively increase lipid peroxidation and synergistically trigger hepatic cytokine expression in a transgenic mouse model. *J. Hepatol.,* 39, 1020-1027 (2003).

Podevin, P., Carpentier, A., Pene, V., Aoudjehane, L., Carriere, M., Zaidi, S., Hernandez, C., Calle, V., Meritet, J. F., Scatton, O., Dreux, M., Cosset, F. L., Wakita, T., Bartenschlager, R., Demignot, S., Conti, F., Rosenberg, A. R., and Calmus, Y., Production of infectious hepatitis C virus in primary cultures of human adult hepatocytes. *Gastroenterology,* 139, 1355-1364 (2010).

Puig, M., Mihalik, K., Tilton, J. C., Williams, O., Merchlinsky, M., Connors, M., Feinstone, S. M., and Major, M. E., CD4+ immune escape and subsequent T-cell failure following chimpanzee immunization against hepatitis C virus. *Hepatology,* 44, 736-745 (2006).

Rambhatla, L., Chiu, C. P., Kundu, P., Peng, Y., and Carpenter, M. K., Generation of hepatocyte-like cells from human embryonic stem cells. *Cell Transplant.*, 12, 1-11 (2003).

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

Ren, S. and Nassal, M., Hepatitis B virus (HBV) virion and covalently closed circular DNA formation in primary tupaia hepatocytes and human hepatoma cell lines upon HBV genome transduction with replication-defective adenovirus vectors. *J. Virol.,* 75, 1104-1116 (2001).

Renard, N., Boucreux, D., Lemonnier, F., and Inchauspe, G., HLA-A2 transgenic mouse model: potential utility for development of an HCV vaccine. *J. Hepatol.,* 32, 363-364 (2000).

Robinet, E. and Baumert, T. F., Host and viral determinants for engraftment of virus permissive human hepatocytes into chimeric immunodeficient mice. *J. Hepatol.,* 53, 421-423 (2010).

Sandgren, E. P., Palmiter, R. D., Heckel, J. L., Daugherty, C. C., Brinster, R. L., and Degen, J. L., Complete hepatic regeneration after somatic deletion of an albumin-plasminogen activator transgene. *Cell*, 66, 245-256 (1991).

Schulze-Bergkamen, H., Untergasser, A., Dax, A., Vogel, H., Buchler, P., Klar, E., Lehnert, T., Friess, H., Buchler, M. W., Kirschfink, M., Stremmel, W., Krammer, P. H., Muller, M., and Protzer, U., Primary human hepatocytes--a valuable tool for investigation of apoptosis and hepatitis B virus infection. *J. Hepatol.,* 38, 736-744 (2003).

Seigneres, B., Aguesse-Germon, S., Pichoud, C., Vuillermoz, I., Jamard, C., Trepo, C., and Zoulim, F., Duck hepatitis B virus polymerase gene mutants associated with resistance to lamivudine have a decreased replication capacity *in vitro* and *in vivo*. *J. Hepatol.,* 34, 114-122 (2001).

Sells, M. A., Zelent, A. Z., Shvartsman, M., and Acs, G., Replicative intermediates of hepatitis B virus in HepG2 cells that produce infectious virions. *J. Virol.,* 62, 2836-2844 (1988).

Shuai, L. F., Tang, B. H., Zhang, R. S., Zhao, Y., Yang, G. Z., and Cheng, X. G., [Establishment of a tight tetracycline-controlled HCV-C double transgenic mouse model]. *Nan*

Fang Yi Ke Da Xue Xue Bao 28, 1530-1533 (2008).

Si-Tayeb, K., Noto, F. K., Nagaoka, M., Li, J., Battle, M. A., Duris, C., North, P. E., Dalton, S., and Duncan, S. A., Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology,* 51, 297-305 (2010).

Sugiyama, M., Tanaka, Y., Sakamoto, T., Maruyama, I., Shimada, T., Takahashi, S., Shirai, T., Kato, H., Nagao, M., Miyakawa, Y., and Mizokami, M., Early dynamics of hepatitis B virus in chimeric mice carrying human hepatocytes monoinfected or coinfected with genotype G. *Hepatology,* 45, 929-937 (2007).

Sullivan, G. J., Hay, D. C., Park, I. H., Fletcher, J., Hannoun, Z., Payne, C. M., Dalgetty, D., Black, J. R., Ross, J. A., Samuel, K., Wang, G., Daley, G. Q., Lee, J. H., Church, G. M., Forbes, S. J., Iredale, J. P., and Wilmut, I., Generation of functional human hepatic endoderm from human induced pluripotent stem cells. *Hepatology,* 51, 329-335 (2010).

Sun, D. and Nassal, M., Stable HepG2- and Huh7-based human hepatoma cell lines for efficient regulated expression of infectious hepatitis B virus. *J. Hepatol.,* 45, 636-645 (2006).

Tabuchi, A., Tanaka, J., Katayama, K., Mizui, M., Matsukura, H., Yugi, H., Shimada, T., Miyakawa, Y., and Yoshizawa, H., Titration of hepatitis B virus infectivity in the sera of pre-acute and late acute phases of HBV infection: transmission experiments to chimeric mice with human liver repopulated hepatocytes. *J. Med. Virol.*, 80, 2064-2068 (2008).

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S., Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 131, 861-872 (2007).

Tanaka, Y., Sanchez, L. V., Sugiyama, M., Sakamoto, T., Kurbanov, F., Tatematsu, K., Roman, S., Takahashi, S., Shirai, T., Panduro, A., and Mizokami, M., Characteristics of hepatitis B virus genotype G coinfected with genotype H in chimeric mice carrying human hepatocytes. *Virology*, 376, 408-415 (2008).

Te, H. S. and Jensen, D. M., Epidemiology of hepatitis B and C viruses: a global overview. *Clin. Liver Dis.*, 14, 1-21, vii (2010).

Thursz, M., Yee, L., and Khakoo, S., Understanding the host genetics of chronic hepatitis B and C. *Semin. Liver Dis.,* 31, 115-127 (2011).

Tomita, T., Yokosuka, O., Tagawa, M., Saisho, H., Tamura, S., Fukuda, I., and Omata, M., Decrease of wild-type and precore mutant duck hepatitis B virus replication during lamivudine treatment in white Pekin ducks infected with the viruses. *J. Hepatol.,* 32, 850-858 (2000).

Tsuge, M., Hiraga, N., Takaishi, H., Noguchi, C., Oga, H., Imamura, M., Takahashi, S., Iwao, E., Fujimoto, Y., Ochi, H., Chayama, K., Tateno, C., and Yoshizato, K., Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis B virus. *Hepatology,* 42, 1046-1054 (2005).

Walter, E., R. Keist, B. Niederost, I. Pult and H. E. Blum, Hepatitis B virus infection of tupaia hepatocytes *in vitro* and *in vivo*. *Hepatology,* 24, 1-5 (1996).

Washburn, M. L., Bility, M. T., Zhang, L., Kovalev, G. I., Buntzman, A., Frelinger, J. A., Barry, W., Ploss, A., Rice, C. M., and Su, L., A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. *Gastroenterology,* 140, 1334-1344 (2011).

Weber, O., Schlemmer, K. H., Hartmann, E., Hagelschuer, I., Paessens, A., Graef, E., Deres, K., Goldmann, S., Niewoehner, U., Stoltefuss, J., Haebich, D., Ruebsamen-Waigmann, H., and Wohlfeil, S., Inhibition of human hepatitis B virus (HBV) by a novel non-nucleosidic compound in a transgenic mouse model. *Antiviral. Res.*, 54, 69-78 (2002).

Wedemeyer, H., Gagneten, S., Davis, A., Bartenschlager, R., Feinstone, S., and Rehermann, B., Oral immunization with HCV-NS3-transformed Salmonella: induction of HCV-specific CTL in a transgenic mouse model. *Gastroenterology,* 121, 1158-1166 (2001).

Yang, J. D. and Roberts, L. R., Hepatocellular carcinoma: A global view. *Nat. Rev. Gastroenterol. Hepatol.,* 7, 448-458 (2010).

Yokosuka, O., Omata, M., and Ito, Y., Expression of pre-S1, pre-S2, and C proteins in duck hepatitis B virus infection. *Virology,* 167, 82-86 (1988).

Youn, J. W., Park, S. H., Lavillette, D., Cosset, F. L., Yang, S. H., Lee, C. G., Jin, H. T., Kim, C. M., Shata, M. T., Lee, D. H., Pfahler, W., Prince, A. M., and Sung, Y. C., Sustained E2 antibody response correlates with reduced peak viremia after hepatitis C virus infection in the chimpanzee. *Hepatology,* 42, 1429-1436 (2005).

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, Slukvin, II, R., and Thomson, J. A., Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 318, 1917-1920 (2007).

Yu, W., Goddard, C., Clearfield, E., Mills, C., Xiao, T., Guo, H., Morrey, J. D., Motter, N. E., Zhao, K., Block, T. M., Cuconati, A., and Xu, X., Design, synthesis, and biological evaluation of triazolo-pyrimidine derivatives as novel inhibitors of hepatitis B virus surface antigen (HBsAg) secretion. *J. Med. Chem.*, 54, 5660-5670 (2011).

Zhang, N., An, M. C., Montoro, D., and Ellerby, L. M., Characterization of human huntington's disease cell model from induced pluripotent stem cells. *PLoS Curr.,* 2, RRN1193 (2010).

Zhao, D., Chen, S., Cai, J., Guo, Y., Song, Z., Che, J., Liu, C., Wu, C., Ding, M., and Deng, H., Derivation and characterization of hepatic progenitor cells from human embryonic stem cells. *PLoS ONE*, 4, e6468 (2009).

Zwi, L., Caspi, O., Arbel, G., Huber, I., Gepstein, A., Park, I. H., and Gepstein, L., Cardiomyocyte differentiation of human induced pluripotent stem cells. *Circulation*, 120, 1513-1523 (2009).