Animal models for studying hepatitis C and alcohol effects on liver

David F Mercer

David F Mercer, Department of Surgery, Liver/Small Bowel Transplant Program, University of Nebraska Medical Center, 983285 Nebraska Medical Center, Omaha, NE 68198-3285, United States

Author contributions: Mercer DF wrote this paper.

Correspondence to: David F Mercer, MD, PhD, Director, Intestinal Rehabilitation Program, Department of Surgery, Liver/Small Bowel Transplant Program, University of Nebraska Medical Center, 983285 Nebraska Medical Center, Omaha, NE 68198-3285, United States. dm Mercer@unmc.ed

Telephone: +1-402-559-6955 Fax: +1-402-559-3434

Received: January 11, 2011 Revised: March 8, 2011 Accepted: March 15, 2011 Published online: May 28, 2011

Abstract

Chronic consumption of ethanol has a dramatic effect on the clinical outcome of patients with hepatitis C virus (HCV) infection, but the mechanism linking these two pathologies is unknown. Presently, in vitro systems are limited in their ability to study the interaction between a productive wild-type HCV infection and chronic ethanol exposure. Mouse models are potentially very useful in dissecting elements of the HCV-ethanol relationship. Experiments in mice that transgenically express HCV proteins are outlined, as are experiments for the generation of mice with chimeric human livers. The latter models appear to have the most promise for accurately modeling the effects of chronic ethanol intake in HCV-infected human livers.

© 2011 Baishideng. All rights reserved.

Key words: Mouse models; Hepatitis C; Ethanol; Transgenic mice

Peer reviewers: Dr. Shivananda Nayak, PhD, Department of Preclinical Sciences, Biochemistry Unit, Faculty of Medical Sciences, The University of The West Indies, Building 36, EWMSC, Mount Hope, Trinidad and Tobago; Naoki Sakata, MD, PhD, Division of Hepato-Biliary Pancreatic Surgery, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan

Mercer DF. Animal models for studying hepatitis C and alcohol effects on liver. World J Gastroenterol 2011; 17(20): 2515-2519

Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i20/2515.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i20.2515

INTRODUCTION

Chronic consumption of ethanol has a dramatic effect on the clinical outcome of patients with hepatitis C virus (HCV) infection. HCV-infected patients who also abuse alcohol have higher levels of HCV RNA, accelerated progression of fibrosis, and clinical disease, and an overall increased risk for development of hepatocellular carcinoma (HCC). Although both chronic alcohol use and HCV infection are individually injurious to the liver, when combined, their effects seem to be multiplied. Despite the well-known deleterious consequences of this combination, however, the mechanism that links these two pathologies remains obscure.

Complicating the study of HCV and ethanol is the fact that in vitro systems that support viral replication are based on the HCV strain JFH-1, a genotype 2a virus that caused an acute self-limited viral hepatitis in a young Japanese male. Although unquestionably valuable in advancing the field of HCV biology, care must be taken in extrapolating results from this unique viral strain into wild-type strains in the community. Furthermore, the hepatoma-derived cell lines that are capable of supporting HCV replication, such as Huh-7, are inherently deficient in main ethanol-metabolizing enzymes such as CYP2E1 and alcohol dehydrogenase, although this has been overcome in part through the engineering of cell lines to metabolize ethanol and support HCV replication.

As pointed out by McCartney and Beard, progress
in this area has been significantly hampered by lack of a small animal model system. Species-restriction of HCV to humans and chimpanzees has impaired the ability to study HCV and ethanol use in vivo. Researchers have attempted to overcome this block through the development of transgenic strains of mice that express key portions of the HCV genome, and through the creation of mice with chimeric human livers. In this review, we explore the studies in transgenic systems, and examine humanized mouse models as potential platforms for HCV/ethanol studies.

**TRANSGENIC MOUSE SYSTEMS**

Transgenic mice that express portions of or the entire HCV genome have been created, and some strains have been used in experiments that have explored the relationship between ethanol administration and viral protein expression. In core-expressing mice exposed to 5% ethanol feeding for 3 wk, total reactive oxygen species were significantly elevated as compared to control animals. In the absence of ethanol, these same mice showed activation of the mitogen-activated protein kinase pathway (which led to enhanced cellular proliferation signaling), which was significantly enhanced by the addition of 3 wk ethanol. These murine experiments suggest that hepatocarcinogenesis mediated through the expression of HCV core protein is enhanced by medium-term administration of ethanol, and that this model system is appropriate for assessing the core protein/ethanol interaction.

In another series of experiments that have examined the HCV/ethanol relationship, core-expressing mice were subjected to chronic ethanol feeding (20% ethanol for 10 mo), and examined for effects on lipid oxidation and peroxidation, hepatic lipoprotein secretion or cytokine expression. No interaction was seen between core expression and ethanol ingestion for lipid oxidation or secretion of lipoproteins, but an additive effect was seen on lipid peroxidation and a synergistic effect on expression of hepatic transforming growth factor-β and tumor necrosis factor-α. The latter effect mimics the accelerated fibrosis that is seen in HCV-infected patients who abuse alcohol, and supports the validity of a core-expressing transgenic mouse model for long-term experiments.

The interaction between NS5a expression and ethanol ingestion in carcinogenesis has been explored in an elegant study by Machida et al., who have used mice on a C57BL/6 background that transgenically expressed NS5a with either wild-type or knocked-out Toll-like receptor (TLR4) expression. When fed ethanol by intragastric infusion for 4 wk, alcoholic steatohepatitis was significantly increased in NS5a-expressing mice compared with non-expressing controls; an effect that was largely enhanced by the addition of 3 wk ethanol. These murine experiments suggest that hepatocarcinogenesis mediated through the expression of HCV core protein is enhanced by medium-term administration of ethanol, and that this model system is appropriate for assessing the core protein/ethanol interaction.

**CHIMERIC MOUSE MODELS**

Given the species-restriction of HCV and the general inability to infect and maintain primary human hepatocytes in culture reliably, researchers have turned to alternate approaches to develop a model that is capable of supporting HCV infection in vivo. The establishment of murine models that support engraftment and expansion of non-transformed human hepatocytes within the liver has led to the term “chimeric mice”, which here refers to mice with livers that are composed of substantial numbers of human hepatocytes. Two separate models, the Alb-uPA and the FAH-deficient mouse, appear to be capable of sustained support of human liver cells, and demonstrate many properties that make them useful for the study of HCV and ethanol.

**Alb-uPA model**

The first success in this area was the development of the SCID/Alb-uPA mouse. The Alb-uPA transgene is a tandem array of murine urokinase genes under the control of the albumin promoter, which target overexpression of urokinase to the murine liver in utero and after birth. Expression of the transgene causes a bleeding diathesis and hepatic toxicity, and produces a chronic stimulus for regeneration to which the mouse is incapable of responding. After spontaneous somatic deletion of portions of the transgene, cells are no longer restrained by expression of the transgene, and rapidly proliferate to fill the liver with non-transgenic cells, which reverses the liver and bleeding defects. By transplanting either mouse or rat hepatocytes into the portal venous system, these findings suggest that NS5a expression and ethanol ingestion affect hepatic inflammation and carcinogenesis, which are mediated through the TLR4 pathway. In similar experiments using 12-mo ethanol feeding in NS5a-expressing mice (wild-type TLR4), upregulation of oncogenic pathways such as RNA pol III dependent transcription and TBP and Brf1 expression were induced in animals that were chronically fed alcohol. Taken together, these studies support a role for chronic ethanol use enhancing inflammation and carcinogenesis in livers that express NS5a.

Although they are seemingly valid and useful for studying the interaction between virally expressed proteins and ethanol ingestion, it is important to stress that transgenic models are not models of infection, and the expression of viral proteins is not under the same controls as would be seen in naturally infected hepatocytes. Although some investigators have demonstrated that expression of viral proteins is similar to that seen in human tissue sections, the expression is indiscriminate in all hepatocytes, which differs from the variable regions of replication seen in human liver. Additionally, the intracellular location of expression might differ from that in wild-type infections. Transgenic models are undoubtedly useful, but cannot yet evaluate the interaction of ethanol and HCV within the context of a full viral reproductive cycle.

These findings suggest that NS5a expression and ethanol ingestion affect hepatic inflammation and carcinogenesis, which are mediated through the TLR4 pathway. In similar experiments using 12-mo ethanol feeding in NS5a-expressing mice (wild-type TLR4), upregulation of oncogenic pathways such as RNA pol III dependent transcription and TBP and Brf1 expression were induced in animals that were chronically fed alcohol. Taken together, these studies support a role for chronic ethanol use enhancing inflammation and carcinogenesis in livers that express NS5a.
produced that is capable of supporting expansion of the Alb-uPA phenotype\[21,22\].

Mice from the Alb-uPA strain have been crossed with an immunodeficient strain (c.b17-SCID-bg) and the transgene bred to homozygosity. These mice can then be transplanted intrasplenically with human hepatocytes, and have been shown by multiple groups to be capable of supporting high levels (up to 90%) of human chimerism within the liver\[18,23,24\]. In mice with sufficient human chimerism (typically > 20%), after inoculation with HCV, infections are established at levels identical to those seen in infected humans, and the infected state persists to beyond 16 wk after inoculation, often to the life of the infected animal\[18\]. The virus can be serially passaged between mice, which confirms that fully formed and infectious particles are produced, and the mice are capable of being infected with virus passaged through cell culture. Infections have been successfully established using viral genotypes 1a, 1b, 2a, 3a, 4a, and 6. The system has been confirmed by multiple groups to model accurately, replication, packaging and release of infectious particles\[25\], respond to human interferon α2b, β, putative antiviral agents\[27,28\], and blockade of infection by passive immunization\[29\].

Important in the study of ethanol-HCV interactions is the similarity between chimeric mouse and normal human liver metabolism. Similarities in the genomic response to HCV infection between human and chimeric mouse livers have been demonstrated by Walters et al\[30\], which suggests that not only does the system support the viral life cycle, but it also models the normal human response. In a series of experiments by a Japanese group, it has been shown that chimeric mouse livers express a wide variety of mRNA for drug-metabolizing enzymes and transporters\[31\], and that the levels of protein expression of these enzymes are very similar to those from source human liver tissue\[32\]. The expression of specific cytochrome P450 enzymes has also been studied, and shown to be appropriately expressed and induced (CYP3A4\[33\]), and inhibited (CYP2D6\[34\]). Although there have been no published studies on the metabolism of ethanol in chimeric livers, generalizing from other enzymes systems, it would appear likely to be very similar to that in humans.

**FAH-deficient model**

An alternate model of repopulation has been developed based on mice rendered deficient in the tyrosine catabolic enzyme fumarylacetoacetate hydratase (FAH)\[35\]. FAH mutant mice are protected by administration of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) in their water source, and develop liver disease when it is withdrawn, hence allowing for conditional expression of the phenotype. After withdrawal of NTBC, there is a stimulus for proliferation that can be exploited to expand a population of transplanted hepatocytes, similar to what happens in the Alb-uPA model. By crossing the FAH-deficient strain onto an immunodeficient background (Rag2/ common γ-chain knockout), a strain of mice has been produced that is capable of supporting expansion of human hepatocyte grafts\[36\]. This model requires temporary introduction of the uPA gene via an adenoviral vector\[37\] to initiate engraftment. This model has the advantage of being useful for transplantation at any age (Alb-uPA mice are typically transplanted between days 7 and 28 of life), however, usable engraftment (serum human albumin level > 1 mg/mL) was achieved in only seven of 43 transplants (16%); somewhat lower than that seen in the Alb-uPA model. However, very high level engraftment has been achieved in some cases.

Chimeric FAH-deficient mice have been shown to express drug-metabolizing genes (CYP1A2, CYP3A4) at levels typical of adult human liver, and when hepatocytes have been isolated from chimeric livers and plated in temporary cultures, they have been found to be indistinguishable from primary human hepatocytes in standard drug metabolism assays\[38\]. In experiments by another group, these chimeric mice have been shown to be capable of supporting HCV infection, and of responding to standard antiviral therapies, including pegylated interferon (peg-IFN), peg-IFN plus ribavirin, and the cyclophilin inhibitor Debio 025\[39\]. Based on these experimental findings, it appears that chimeric FAH-deficient mice should also be appropriate for the modeling of HCV/ethanol interactions in the human liver.

**Pilot studies of HCV and ethanol in Alb-uPA mice**

We have conducted preliminary studies on the feasibility of using chimeric mice in the study of HCV/ethanol interactions, based on the Alb-uPA model. Potential concerns about tolerability of an ethanol regimen, as well as the ability to model the human response to ethanol exposure have been addressed by feeding a cohort of chimeric mice a diet including 20% ethanol in water for 5 wk. These mice tolerated the ethanol protocol with a slight decrease in weight and fluid consumption, as compared with mice on a control diet, and no evidence of increased mortality. At completion of ethanol feeding, liver samples from two of these mice were taken and analyzed by HPLC for glutathione (GSH) and SAM levels. These samples demonstrated a 40% decrease in SAM levels and an 83% decrease in GSH levels; both indicative of chronic ethanol toxicity on the livers. Studies of ethanol administration in HCV-infected mice are ongoing.

**CONCLUSION**

Based on the state of knowledge presently available, study of the complete interaction between ethanol and natural HCV infection will require the use of mouse models. Transgenic models have proven useful to study the relationship between ethanol exposure and viral protein expression, but have limitations in how accurately they can model HCV infections in humans. Studies based on chimeric mice appear to be the most promising, but have their own complexities which include technical challenges in establishing these models and the ability to extract the human response of a chimeric liver from within its murine...
background. However, in well-engrafted mice, the overall response seems to mimic that of humans so closely that the murine background might not matter.

REFERENCES

1 Poyntard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997; 349: 825-832

2 Corrao G, Arico S. Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. Hepatology 1998; 27: 914-919

3 Seef LB, Buskell-Bales Z, Wright EC, Durako SJ, Alter HJ, Iber FL, Hollinger FB, Gitnick G, Knodell RG, Perrillo RP. Long-term mortality after transfusion-associated non-A, non-B hepatitis. The National Heart, Lung, and Blood Institute Study Group. N Engl J Med 1992; 327: 1906-1911

4 Aizawa Y, Shibamoto Y, Takagi K, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. Cancer 2000; 89: 53-59

5 Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, Decarli A, Trevisi P, Ribero ML, Martelll C, Porru S, Nardi G. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002; 155: 323-331

6 Wakita T, Pletschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005; 11: 791-796

7 Kato T, Furuokaka A, Miyamoto M, Date T, Yasui K, Hiramoto J, Nagayama K, Tanaka T, Wakita T. Sequence analysis of hepatitis C virus isolated from a fulminant hepatitis patient. J Med Virol 2001; 64: 333-339

8 McCartney EM, Semendric L, Helbig KJ, Hinze J, Jones B, Weinman SA, Beard MR. Alcohol metabolism increases the replication of hepatitis C virus and attenuates the antiviral action of interferon. J Infect Dis 2008; 198: 1766-1775

9 McCartney EM, Beard MR. Impact of alcohol on hepatitis C virus replication and interferon signaling. World J Gastroenterol 2010; 16: 1337-1343

10 Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, Okuda M, Gosert R, Xiao SY, Weinman SA, Lemon SM. Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology 2002; 122: 352-365

11 Moriya K, Fuji H, Shintani Y, Yotsuyangi H, Tsutsui T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 1999; 4: 1065-1067

12 Naas T, Ghorbani M, Alvarez-May A, Lapner M, Kothary R, De Repentigny Y, Gomes S, Babiliu K, Giulivi A, Soare C, Azizi A, Diaz-Mitoma F. Characterization of liver histopathology in a transgenic mouse model expressing genotype 1a hepatitis C virus core and envelope proteins 1 and 2. J Gen Virol 2005; 86: 2185-2196

13 Moriya K, Nakagawa K, Santa T, Shintani Y, Fuji H, Miyoshi H, Tsutsui T, Miyazawa T, Ishibashi K, Horie T, Imai K, Todoroki T, Kimura S, Koike K. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 2001; 61: 4365-4370

14 Tsutsui T, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, Matsuura Y, Koike K, Miyamura T. Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. Hepatology 2003; 38: 820-828

15 Perlemuter G, Letteron P, Carnot F, Zavala F, Pessayre D, Nalpas B, Brechet 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 2003; 39: 1020-1027

16 Machida K, Tsukamoto H, Mkrtychian H, Duan L, Dynnyk A, Liu HM, Asahina K, Govindarajan S, Ray R, Ou JH, Seki E, Deshaies R, Miyake K, Lai MM. Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog. Proc Natl Acad Sci USA 2009; 106: 1548-1553

17 Zhou S, Machida K, Tsukamoto H, Johnson DL. Alcohol induces RNA polymerase III-dependent transcription through e-Jun by co-regulating TATA-binding protein (TBP) and Brf1 expression. J Biol Chem 2011; 286: 2393-2401

18 Mercer DF, Schiller DE, Elliott JF, Douglas DN, Hao C, Rinfett A, Addison WR, Fischer KP, Churchill TA, Lakey JR, Tyrrell DL, Ketnetman NM. Hepatitis C virus replication in mice with chimeric human livers. Nat Med 2001; 7: 927-933

19 Heckel JL, Sandgren EP, Degen JL, Palmeri RD, Brinster RL. Neonatal bleeding in transgenic mice expressing urokinase-type plasminogen activator. Cell 1990; 62: 447-456

20 Sandgren EP, Palmeri RD, Heckel JL, Daugherty CC, Brinster RL, Degen JL. Complete hepatic regeneration after somatic deletion of an albumin-plasminogen activator transgene. Cell 1991; 66: 245-256

21 Rhim JA, Sandgren EP, Degen JL, Palmeri RD, Brinster RL. Replacement of diseased mouse liver by hepatic cell transplantation. Science 1994; 263: 1149-1152

22 Rhim JA, Sandgren EP, Palmeri RD, Brinster RL. Complete reconstitution of mouse liver with xenogeneic hepatocytes. Proc Natl Acad Sci USA 1995; 92: 4942-4946

23 Meuleman P, Libbrecht L, De Vos R, de Hemptinne B, Gevaert K, Vandekerckhove J, Roskams T, Leroux-Roels G. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. Hepatology 2005; 41: 847-856

24 Tateno C, Yoshizato Y, Saito N, Kataoka M, Utoh R, Yamaaki C, Tachibana A, Soeno Y, Asahina K, Hino H, Asahara T, Yokoi T, Furukawa T, Yoshizato K. Near completely humanized liver in mice shows human-type metabolic responses to drugs. Am J Pathol 2004; 165: 901-912

25 Lindendach BD, Meuleman P, Ploss A, Vanvolleghem T, Syder AJ, McKeating JA, Landford RE, Feinstein SM, Major ME, Leroux-Roels G, Rice CM. Cell culture-grown hepatitis C virus in vivo. Proc Natl Acad Sci USA 2006; 103: 3805-3809

26 Hiraga N, Imamura M, Tsuge M, Noguchi C, Takahashi S, Iwao E, Fujimoto Y, Abe H, Maekawa T, Ochi H, Tateno C, Yoshizato K, Sakai A, Sakai Y, Honda M, Kaneko S, Wakita T, Chayama K. Infection of human hepatocyte chimeric mouse with genetically engineered hepatitis C virus and its susceptibility to interferon. Hepatology 1997; 26: 1346-1353

27 Ketnetman NM, Weiner AJ, O’Connell J, Collett M, Gao T, Aukerman L, Kovelsky R, Ni ZJ, Zhu Q, Hashash A, Kline J, Hsi B, Schiller D, Douglas D, Tyrrell DL, Mercer DF. Anti-HCV therapies in chimeric scid-Ali/uPA mice parallel outcomes in human clinical application. Hepatology 2006; 43: 1346-1353

28 Ketnetman NM, Howe AY, Gao T, Lewis J, Pevear D, Lund G, Douglas D, Mercer DF, Tyrrell DL, Immerrmann F, Chaudhary I, Speith J, Villano SA, O’Connell J, Collett M. HCV796: A selective nonstructural protein 5B polymerase inhibitor with potent anti-hepatitis C virus activity in vitro, in mice with chimeric human livers, and in humans infected with hepatitis C virus. Hepatology 2009; 49: 745-752

29 Meuleman P, Hesselgesser J, Paulson M, Vanvolleghem T, Desombre I, Reiser H, Leroux-Roels G. Anti-CD81 antibodies can prevent a hepatitis C virus infection in vivo. Hepatology 2008; 48: 1761-1768
Walters KA, Joyce MA, Thompson JC, Smith MW, Yeh MM, Proll S, Zhu LF, Gao TJ, Kneteman NM, Tyrrell DL, Katze MG. Host-specific response to HCV infection in the chimeric SCID-beige/Alb-uPA mouse model: role of the innate antiviral immune response. *PLoS Pathog* 2006; 2: e59

Nishimura M, Yoshitsugu H, Yokoi T, Tateno C, Kataoka M, Horie T, Yoshizato K, Naito S. Evaluation of mRNA expression of human drug-metabolizing enzymes and transporters in chimeric mouse with humanized liver. *Xenobiotica* 2005; 35: 877-890

Katoh M, Matsui T, Okumura H, Nakajima M, Nishimura M, Naito S, Tateno C, Yoshizato K, Yokoi T. Expression of human phase II enzymes in chimeric mice with humanized liver. *Drug Metab Dispos* 2005; 33: 1333-1340

Katoh M, Watanabe M, Tabata T, Sato Y, Nakajima M, Nishimura M, Naito S, Tateno C, Iwasaki K, Yoshizato K, Yokoi T. In vivo induction of human cytochrome P450 3A4 by rifabutin in chimeric mice with humanized liver. *Xenobiotica* 2005; 35: 863-875

Katoh M, Sawada T, Soeno Y, Nakajima M, Tateno C, Yoshizato K, Yokoi T. In vivo drug metabolism model for human cytochrome P450 enzyme using chimeric mice with humanized liver. *J Pharm Sci* 2007; 96: 428-437

Grompe M, al-Dhalimy M, Finegold M, Ou CN, Burlingame T, Kernaway NG, Soriano P. Loss of fumarylacetoacetate hydratase is responsible for the neonatal hepatic dysfunction phenotype of lethal albino mice. *Genes Dev* 1993; 7: 2298-2307

Azuma H, Paulk N, Ranade A, Dorrell C, al-Dhalimy M, Ellis E, Strom S, Kay MA, Finegold M, Grompe M. Robust expansion of human hepatocytes in Fah-/-/Rag2-/-/Il2rg-/- mice. *Nat Biotechnol* 2007; 25: 903-910

Lieber A, Vranken Peeters MJ, Meuse L, Fausto N, Perkins J, Kay MA. Adenovirus-mediated urokinase gene transfer induces liver regeneration and allows for efficient retrovirus transduction of hepatocytes in vivo. *Proc Natl Acad Sci USA* 1995; 92: 6210-6214

Bissig KD, Wieland SF, Tran P, Isogawa M, Le TT, Chisari FV, Verma IM. Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. *J Clin Invest* 2010; 120: 924-930