gradually and steadily replaced the uPA-damaged host hepatocytes and occupied ~ 90% of the whole hepatocytes ~ 6 weeks post-transplantation. Although the repopulated liver has not been characterized in detail, this m-model could be utilized to generate hepatocyte-*h*-mice without the problems related to constitutive overexpression of uPA.

Major urinary protein (MUP) genes are expressed at high levels in hepatocytes in mice and are considered to be involved in binding pheromones or other small molecules, thereby facilitating their secretion and excretion, and their expression does not begin until mice are 2 to 4 weeks old [66]. This suggests the usefulness of their promoter sequences for driving the expression of uPA gene specifically in hepatocytes of mice that are older and more mature compared to the expression driven by the Alb promoter/enhancer. Thus, it is expected that uPA-mediated neonatal lethality could be eliminated in this model [67] and that the time window for hu-hepatocytes is more flexible, which permits more efficient repopulation by donor cells. Considering these advantages, MUP-uPA-Tg mice were generated and characterized [67]. As predicted, the expression of uPA was significantly delayed in some lines of the Tg-mice and was used for transplantation experiments of normal healthy m-hepatocytes. The donor hepatocytes were transplanted between 2 and 7 weeks of recipient age, and recipient mice were sacrificed 8 weeks later. The repopulation ranged up to 80% in young recipients. Some recipient mice homozygous for the MUP-uPA transgene displayed 93% repopulation by donor cells. It remains to be studied whether these mice could be utilized as hosts to generate hepatocyte-*h*-mice.

There are practical disadvantages in using triply mutated $Fah^{-/-}/Rag^{2-/-}/Il2rg^{-/-}$ mice as immunodeficient hosts, including the high mortality during colony breeding and cell transplantation surgery. These disadvantages motivated a research group to utilize doubly mutated $Fah^{-/-}/Rag^{2-/-}$ as hosts, which had lower mortality rates (~ 60%) during colony breeding than $Fah^{-/-}/Rag^{2-/-}/Il2rg^{-/-}$ mice [68]. The research group fortified their low xeno-repopulation capacity due to the intactness of NK cells with two known pharmacological immunosuppressors, anti-asialo GM1 and FK506, the latter of which is also known to induce hepatocyte proliferation [69]. The $Fah^{-/-}/Rag^{2-/-}$ mice were given anti-asialo GM1 and FK506 by intraperitoneal injection and through drinking water, respectively. The single treatment with anti-asialo GM1 was quite effective in decreasing NK cells (~ 6% of the original level). The chimerism of anti-asialo-GM1-treated $Fah^{-/-}/Rag^{2-/-}$ mice ranged from ~ 3 to 32% at 12 weeks post-hu-hepatocyte-transplantation, which was still lower than those of $Fah^{-/-}/Rag^{2-/-}/Il2rg^{-/-}$ recipients. The combined treatment of both increased the repopulation to as high as ~ 67% (ranging from ~ 10 to 67%) at 12 weeks post-transplantation, which was comparable to that of $Fah^{-/-}/Rag^{2-/-}/Il2rg^{-/-}$ mice. The repopulated hu-hepatocytes recovered the metabolic functionality and showed infectivity against HBV. However, this model has still not obtained high levels of repopulation, and phenotypes of its hu-hepatocytes are required to be sufficiently characterized in relation to those in the hu-liver.

5. Hepatocyte and immune cell dually humanized mouse

The hepatocyte-*h*-mouse has been proven to be useful for predicting metabolic patterns of drugs in hu-liver and for investigating infection and propagation of HCV/HBV. However, the currently available chimeric mouse cannot be a liver disease model for drug/hepatitis-induced inflammation, fibrogenesis and hepatocytic carcinogenesis due to the lack of hu-cells responsible for inflammatory and immune reactions. Therefore, it is an important challenge to create the hepatocyte-*h*-mouse, which can be used as a convenient tool for developing drugs/medicines that suppress the initiation of liver diseases or that eliminate the diseased liver, which are the *bona fide* achievements of medical and pharmaceutical treatments in hospitals. Generally, the immune system functions as a major network for protecting the body from pathogens and deleterious drugs by detecting exogenous entities and inducing inflammatory reactions. Thus, it is logical that researchers endeavor to solve the limitations of the first-generation hepatocyte-*h*-m-model by reconstructing the hu-immune system in the hepatocyte-*h*-mouse [70].

Although it is known that T cells, in particular $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells (CTL), play prime roles in HCV control and clearance [71], very little is understood about the immune response to the viral infection, which led a research group to undertake a study to generate a mouse engrafted with hu-liver cells and a functional hu-immune system [72]. A suicide gene and hu-embryonic liver cells were utilized to delete the host (m-) hepatocytes and to humanize both hepatocytes and immune cells, respectively. Caspase 8 is a member of the cystein-aspartic acid protease (caspase) family, which is involved in apoptotic alterations of cells induced by Fas and various apoptotic stimuli. Caspases exist as inactive proenzymes composed of large and small protease subunits. The activation of the proenzyme requires its cleavage to each of the subunits and their dimerization. FKBPv [Phe36Val mutant FKBP (FK506 binding protein)/AP20187 (a FK1012 analog)] has been utilized as a 'dimerization agent' [73]. AP20187, a prodrug, binds to FKBPv with ~ 1000-fold greater affinity than to endogenous FKBP and dimerizes it. The cited research group made a gene construct (AFC8 gene) by fusing the catalytic domains of hu-caspase 8 to FKBPv, which induces conditional apoptosis of a targeted cell type as originally reported by Pajvani *et al.* [74]. In this case, the construct was designed such that it is driven by the Alb promoter/enhancer for targeted expression in hepatocytes. The hu-hepatocyte progenitor cells and $CD34^+$ cells as hematopoietic stem cells were each isolated from 15 – 18-week-old fetal liver tissues, the purity being > 95%, and both were co-injected into the liver of < 5-day-old newborn immunodeficient Tg-Rag2-γC-null mice with the AFC8 transgene that

had been preconditioned by irradiation with X-ray. Then, the mice were treated with AP20187 to induce apoptosis in the host hepatocytes. The hu-Alb$^+$-hepatocytes were significantly increased in the liver as expected, and they expressed CYP2E1, CYP2C9 and UGT2B7, although the average RI was lower (~ 15% between 5 and 16 weeks after transplant) than those of the conventional hu-hepatocyte chimeric mice. As predicted, these mice included hu-immune cells in all lymphoid organs, including the liver, indicating that they were doubly *h*-mice in terms of their liver and immune system. However, functional hu-B cells were reported not to be present, indicating that this model is on the way of technical completion, because antibodies have been shown to play important roles in the pathogenesis of HCV infection and viral persistence [75].

To examine the infectivity toward HCV, AFC8 mice were inoculated with patient HCV isolates (genotype 1a), and their livers were examined for the presence of HCV genomic RNA 1 – 4 months post-infection. Approximately 50% showed its presence with variable levels, although not in the blood, which is most likely due to a low repopulation rate of hu-hepatocytes. In addition to the viral infection, hu-immune cells infiltrated into the liver of HCV-infected AFC8 mice, including CD45$^+$ leucocytes, CD68$^+$ macrophages, CD3$^+$ T cells and other multiple hu-leukocyte subsets, although functional B cells were not present as described above. ALT levels were elevated in HCV-infected mice, but not in HCV-non-infected control mice. These facts together strongly suggest that hu-immune cells are actively involved in the process of liver injury, a state of HCV-induced hepatitis that has not been reproduced in the first-generation chimeric mouse, the immunodeficient mouse singly humanized for hepatocytes. In this process, hu-T cells were thought to play primary roles as predicted from clinical data [71], because CD4$^+$ T cells or CD8$^+$ T cells isolated from HCV-infected mice express higher levels of effector cytokines, such as IL2, IFNγ and TNFα, when stimulated *in vitro* with phytohemagglutinin (PHA) than those from non-infected mice. Importantly, HCV-infected AFC8 mice with hu-hepatocytes and hu-immune cells developed severe fibrosis throughout the liver parenchyma as indicated by the appearance of αSMA$^+$-cells (activated stellate cells), but this event did not occur in those with hu-immune cells or hu-hepatocytes alone. Interestingly, the livers of these mice showed elevated mRNA expressions of hu-COL1A1 and TIMP1, but not their m-counterparts. This observation suggests that the hu-hepatic stellate cell progenitors present in the hu-hepatocyte progenitor fraction from 15- to 18-week-old fetal liver tissues developed into mature hu-stellate cells in the host liver and were activated by HCV proteins released from HCV-infected hu-hepatocytes, which supports the notion that HCV infection causes the activation of the hepatic stellate cells, leading to hepatic fibrosis [76]. It is also considered that host (m-) stellate cells are not involved in the fibrogenesis induced by HCV infection of hu-hepatocytes due to the lack of pathophysiological interactions between hu-hepatocytes and m-stellate cells. Similar species-specific interactions between hepatocytes and stellate cells were reported in liver growth of hepatocyte *h*-uPA/SCID mice [14,77]. This pioneering work is undoubtedly a promising first step for further development of a m-model permissive for not only HV infection but also for pathogenesis triggered by interactions between HV and hu-immune cells. Several improvements remain to be made on this dually chimeric m-model. HCVs were variably detected at low levels in liver tissues but not in the blood, which might be due to insufficient RIs (~ 10 – 30%) of the donor cells, as prior studies have reported a high level of HCV infection among chimeric mice with > 50% RI [64,78]. The livers of AFC8 mice are considered to have acute injury, whereas those of the first-generation chimeric mice with high RIs have chronic injury induced by uPA [17] or Fah [32]. It remains to be clarified whether acute or chronic types of liver injury affect the extent of repopulation of xenogeneic hepatocytes [75].

Generally, xenobiotics induce immuno-inflammatory reactions in host tissues. The first-generation hepatocyte-chimeric mice are immunosuppressed, which hampers researchers from studying the hepatic responses induced by xenobiotics under the immuno-ethio-pathogenic conditions, such as viral hepatitis. This limitation is a critical issue for the first-generation hepatocyte-chimeric mice because, for example, hepatitis viruses act as antigens and induce chronic inflammatory-immune reactions in the hu-body and result in severe diseases unless appropriate treatments are given. The necroinflammatory reaction plays a central role in hepatitis virus elimination. We previously demonstrated that the chimeric mouse with high RIs is an ideal model for investigating the mechanisms of HBV viral replication under conditions close to the human liver *in vivo* and for testing a candidate drug effective in inhibiting the viral infection and replication [58-62]. However, it is apparently not an ideal model for investigating the virus-induced inflammatory reactions due to the lack of hu-blood cells.

Apart from hepatitis viruses, xenobiotics generally induce inflammatory-immune reactions in host tissues when administered to humans. The conventional (first-generation) hepatocyte-chimeric mouse is not an animal model for studying hu-hepatic responses induced by xenobiotics, which proceed under appropriate immuno-ethio-pathogenic conditions. A direct and simple approach to overcome this fault is to introduce hu-inflammatory-immune cells into the SCID mouse [79]. CD8$^+$ T cells and NK cells are thought as major players in the elimination of infected cells. We first transplanted hu-hepatocytes into uPA/SCID mice, obtained mice whose livers were highly repopulated with the donor cells and then infected these mice with HBV. After establishing HBV infection, we additionally injected hu-peripheral blood mononuclear cells (hu-PBMCs) into the mice and analyzed liver pathology and infiltrating hu-immune cells with flow cytometry. As expected, hu-hepatocytes showed severe degeneration only in HBV-infected mice transplanted with

hu-PBMCs, and these HBV-infected hu-hepatocytes were eliminated by NK cells and DCs. This was completely prevented in mice treated with anti-Fas antibodies or by depletion of DCs, but not by depletion of $CD8^+$ CTLs. This study clearly demonstrated that DC-activated NK cells induce massive HBV-infected hepatocyte degeneration through the Fas/FasL system and may indicate new therapeutic implications for acute severe/fulminant hepatitis B. Based on this study, it can be said that the introduction of the inflammation/immune-responsible hu-cells into the first-generation chimeric mouse is a promising approach to overcome limitations that are inherent in the conventional hepatocyte-*h*-mouse.

There was another trial to generate hepatocyte-*h*-mice bearing hu-blood cells, in which immunotolerant hosts were utilized instead of immunosuppressive hosts [80]. This trial was based on the well-established mechanism of development of the immune system, by which auto-reactive T-cell clones are completely deleted from the immune repertoire during fetal development [81]. Chimeric m-embryos were generated using normal (immunotolerant) mouse fetuses or blastocysts as hosts and human cord blood $CD34^+$ cells as donors. To make chimeric fetuses, the uterus was exposed by a single abdominal incision, and the donor cells were intraperitoneally inoculated. The chimeric blastocysts made by microinjecting the donor cells were transferred into foster mothers. The livers were immunohistochemically examined to detect hu-hepatocytes using hu-specific antibodies against hu-hepatocyte-specific antigen (HepPar1), hu-Alb and hu-α-1-antitrypsin, or for determining the expression levels of the hu-Alb gene. These examinations showed that hu-cord blood $CD34^+$ cells were able to integrate into preimmune m-liver and give rise to liver cells expressing hepatocyte markers for at least 4 weeks after birth. Although this study provides a possible approach for generating immunocompetent mice harboring hu-hepatocytes, several important issues remain unanswered. These include the identification of these hu-hepatocyte-like cells as functional true hepatocytes, the extent of repopulation of hu-hepatocytes and the extent of immune competency of the mouse.

6. New technologies to increase the usability of the humanized murine model

In the above sections, we have reviewed the hepatocyte-*h*-m-model as a tool for preclinical testing of medicines and for investigating the mechanisms of hepatic virus infections. Although its usefulness has been increasingly recognized among researchers in the related areas, its shortages limit its robust and general use in pharmaceutical and medical arenas. One such shortage is related to its size. Availability of sufficient amounts of blood samples is preferred for the animal model for drug testing. Currently, researchers are able to utilize hu-hepatocytes provided from commercial sources as donor cells. However, these hu-hepatocytes have problems around donor-to-donor variability such as variable engraftment efficiency. Recent progressions in biotechnology are expected to provide us with new sources of host animals and hu-hepatocytes for drug testing, which could alleviate these issues.

6.1 Rats as a host for a humanized animal model

The mouse has been a frequently utilized animal model to study the biology and pathology of the vertebrate liver because of its appropriate size for experimental handlings, its well-characterized genetic background, the availability of a variety of lines and the applicability of commonly utilized genetic and genomic manipulations. However, the rat has traditionally been a major animal model in the area of pharmacological and pharmaceutical studies for the development of new medicine because the rat has an advantage over the mouse in the availability of sufficient blood samples for investigating the PK of drugs. Abundant data regarding the effectiveness, toxicity and the metabolic profiles of drugs and medicines have accumulated from studies with this murine species. Therefore, generation of a rat model with a *h*-liver has been a goal after the establishment of the basic technology to produce hepatocyte-*h*-m-model, but it has been hampered due to the lack of an appropriate method to create a rat with dysfunctional immune systems. A recently developed novel gene-targeting technology based on utilization of zinc-finger nucleases (ZFNs) solved this difficulty, and it is a useful tool to arbitrarily insert or delete base pairs in a targeted gene [82]. ZFNs are made by fusing a specific DNA binding domain containing the tandem zinc finger motifs with a non-specific cleavage domain of the restriction endonuclease *FokI*. When a ZFN construct is introduced into cells, the ZFNs target genes in a site-specific manner and create double-stranded breaks, which are then repaired via non-homologous end joining – a mutagenic process that results in the insertion/deletion of base pairs. Thus, ZFN technology enables researchers to inactivate a targeted gene in living cells, including rat embryonic cells [83]. A research group led by Serikawa prepared ZFN plasmids to generate rats that lack both $Prkdc^{SCID}$ gene and interleukin-2 receptor γ (*Il2rγ*) gene which were referred to as F344-scid γ rats [84]. The obtained rats showed severe immunocompromised phenotypes including the abolishment of NK cells. The double-KO rats aged 5 years were pretreated with retrosine, a pyrrolizidine alkaloid that is toxic to hepatocytes [85], to damage the host liver and were transplanted with hu-hepatocytes. Successful engraftment and repopulation of hu-hepatocytes were indicated by increase of hu-Alb levels in the blood and appearance of small clusters of hu-CK8/18-positive cells in the liver. Currently, these hepatocyte-*h*-rats have not been sufficiently characterized biologically and pathologically and RIs of hu-hepatocytes are not high enough for practical uses, but these rats could be an promising *in vivo* model for the preclinical testing of new drugs, taking advantage of their easier handling and the

availability of a larger volume of blood samples due to larger body size compared to the m-counterpart.

6.2 iPS cells as a source of human cells for liver humanization

When necessary, hu-hepatocytes are generally obtained in hospitals under ethical and clinical regulations in quite limited amounts for specific clinical and therapeutic purposes, but not for studies and experiments such as drug testing. There are hu-hepatocytes that are available from commercial sources that are authorized to collect specified hepatic biopsies from hospitals under the regulations and the requirements set by authorities. Even in this case, the quantity of available hu-hepatocytes is not abundant, and they are usually expensive. In addition, they display lot-to-lot individual variability such as viability and variable engraftment efficiency. These hu-hepatocytes are mostly refractory to genetic manipulations due to their poor proliferative potential. Rapid developments of technologies to differentiate stem cells or progenitor cells into hepatocyte-like cells are changing these unavoidable and unfavorable situations. Several types of stem cells/progenitor cells were the targets of novel sources of hu-hepatocytes, such as embryonic stem cells (ES cells), bone marrow mesenchymal stem cells, peripheral blood stem cells and iPS cells, among which iPS cells are especially worthy of being commented on herein because they are the most promising and versatile potential sources of hu-hepatocytes for drug testing.

A protocol for generating hu-hepatocytes from hu-iPS cells generally consists of two consecutive procedures: the first is for induction of hu-fibroblasts to hu-iPS cells, and the second is for differentiation of hu-iPS cells to hepatocyte-like cells. Takahashi and Yamanaka first demonstrated that only a few defined factors, that is, Oct3/4, Sox2, c-Myc and Klf4, are able to reprogram the differentiated state of m-embryonic or adult fibroblasts to a undifferentiated state, in which these cells exhibit the morphology and growth properties of ES cells and express ES cell-marker genes. These ES-like cells were dubbed iPS cells [86]. It was also shown that primary, genetically unmodified, diploid hu-fibroblasts (IMR90 fetal fibroblasts) were reprogrammed to ES-like cells by a combination of OCT4, SOX2, NANOG and LIN28 [87]. The second protocol was a procedure in which hu-iPS cells obtained from, for example, foreskin fibroblasts, were induced to differentiate into hepatocyte-like cells with a combination of activin A, BMP4/FGF2, HGF and OSM [88]. The hu-iPS cells were induced into definitive endoderm – the endoderm that forms organs in the embryo itself – by activin A treatment. These cells then acquired hepatic specification under the influence of BMP4/FGF2. HGF treatment caused the cells with hepatic specification to become hepatoblasts, which then differentiate to hepatocyte-like cells when treated with OSM.

The hepatocyte-like cells generated were thought to be 'hu-hepatocyte-like' in that they shared many of the morphological characteristics associated with 'genuine' hepatocytes and expressed the representative hu-hepatocyte biomarkers such as hu-Alb and, importantly, a series of genes encoding Phase I and Phase II enzymes, whose expression is characteristic of a fully differentiated hepatocyte. The hepatocyte-like cells secreted hu-Alb in the medium and displayed several hepatic functions, including accumulation of glycogen, metabolism of indocyanine green, accumulation of lipid, active uptake of low density lipoprotein and synthesis of urea. When injected into liver lobes of newborn mice, these hepatocyte-like cells formed hu-Alb-positive foci throughout the injected lobe, indicating that they exhibit the inherent capacity to integrate into the hepatic parenchyma *in vivo*. However, they do not appear to entirely replicate mature liver functions, because the expression levels of hepatocyte-specific enzymes were lower in most cases compared with adult liver samples. Utilizing hu-iPS cell lines generated from the hu-embryonic lung fibroblast cell line MCR5, Takayama *et al.* showed that FoxA2 and HNF1a are useful in generating metabolically functional hepatocytes [89].

Hepatic viral permissiveness will currently be one of the most important issues for the iPS cell-derived hu-hepatocytes, as it was for the hepatocyte *h*-chimeric mouse. Several attempts have been reported to optimize requirements for differentiating hu-iPS cells into functional mature hepatocytes *in vitro* in relation to viral permissiveness. There have been a few studies that demonstrated that iPS cell-derived hu-hepatocytes are permissive to the viral infection *in vitro*. The hu-iPS cells were produced from foreskin fibroblasts, differentiated to hepatocyte-like cells as described above [88] and challenged with genotype 2a HCV with Gaussia luciferase reporter gene [90]. The results were remarkable, showing that the hepatocyte-like cells not only allowed HCV to enter and replicate but also allowed HCV to escape to the culture medium as infectious virions, indicating that iPS cell-derived hu-hepatocyte-like cells are a promising experimental tool to replicate the entire viral life cycle *in vitro* of at least genotype 2a. Furthermore, these cells exhibited antiviral inflammatory responses on HCV-infection by upregulating the expression of genes such as IFN γ-inducible protein 10 (CXCL-10), IFN-inducible T-cell, a chemoattractant (CXCL-10), TNF-α and IL-28B (a member of type III IFN [IFN-l]). These HCV-dependent expressions of innate antiviral cytokine genes could provide iPS cell-derived hu-hepatocyte-like cells with an advantage as an *in vitro* experimental tool for HCV infection and replication over the hu-hepatoma cell line Huh-7 described above. There was also a study that demonstrated the permissiveness of hu-iPS cell-derived hepatocyte-like cells to HCV [91]. Therefore, iPS cell-derived hu-hepatocyte-like cells could be a novel cell culture model for investigating the entire life cycle of HCV, including inflammatory responses to the viral infection, and could contribute to studies on the relationships between host genetics and viral pathogenesis.

Primary hu-fetal liver cell cultures seem to exhibit a similar permissiveness to HCV as iPS cell-derived hepatocytes [92]. A hepatocyte-enriched fraction was prepared from hu-fetal

livers at 16 – 24 week of gestation by collagenase digestion and centrifugation, cultured and used as primary liver cultures for HCV-infectivity tests. HCV replication in these cells was less robust than that in the Huh-7 line. Analyses of gene expression that are associated with IFN-induction and signaling showed that cell culture-produced HCV robustly activated expression of CXCL10 and CXCL11, variably activated IFN-λ (IL-29 and IL-28B) and ISGs such as viperin, IFITM1 and IFIT2, but did not activate IFN-β and TNF-α. These results suggest that innate antiviral mechanisms (especially INF-λ-associated pathways) in primary cells limit HCV replication or spread, which could explain a less robust viral replication in these cells than in Huh-7 lines. However, inductivity of IFN-λ and ISGs by HCV implies that HCV infection of fetal hu-liver cell cultures may provide a useful model for the study of gene induction by HCV *in vivo*, as does the infection of iPS cell-derived hepatocyte-like cells. In addition, the apparent commonness in the induction of antiviral IFN-related cytokines on HCV infection between fetal hu-hepatocytes and hu-iPS cell-derived hepatocyte-like cells suggests an association between their plausible immaturity and permissibility of HCV. It is well known that, paradoxically, sufficiently differentiated primary hepatocytes are generally reluctant to allow HCV to replicate *in vitro*. The hu-iPS cells are thought to be useful as a source of hu-hepatocytes for both *in vitro* and *in vivo* studies. Currently available hepatocyte-*h*-chimeric mice are those with genuine hu-hepatocytes in the liver. It is quite plausible that *h*-chimeric mice bearing hu-iPS cell-derived hepatocyte-like cells in the liver will be generated in the near future and these mice can be utilized as preclinical test animal model.

Regenerative capabilities of m-hepatocyte-like cells that had been differentiated *in vivo* from m-iPS cells were evaluated utilizing genetically FAH (a tyrosine-degrading enzyme)-deficient mice as an experimental model for liver regeneration, which causes the liver diseases, such as tyrosinemia type I [93]. FAH-deficient mice in a pure C57BL/6 background were bred with C57/6 R26R to label the host blastocyst-derived cells in the FAH-deficient neonates. These mice suffer from liver failure in the neonatal period unless treated with NTBC [32,94] as described above. The iPS cells were generated by repeated transfection of m-embryonic fibroblasts with Oct4, Sox2, Kif4 and c-Myc, and they were injected into blastocysts obtained from FAH-deficient mice. The foster mothers bearing these blastocysts were treated with NTBC during pregnancy. When the neonates were 6 days old, the foster mothers were deprived of NTBC. The contribution of iPS cell-derived cells (iPS-hepatocytes) was determined using PCR amplification of the Fah allele in genomic DNA in the digits and livers. Pups derived from FAH-deficient blastocysts that had not been injected with iPS cells rapidly deteriorated. However, those injected with iPS cells and with high levels of digital chimerism continued to thrive. They showed various levels of liver repopulation with FAH^+-hepatocytes, reaching ~ 100% repopulation at 70 days after birth. These FAH^+-hepatocytes were negative to Rosa26 reporter staining, indicating that they resulted from direct iPS cell differentiation, but not from fusion of the iPS cell progeny with blastocyst-derived host hepatocytes.

These mice were challenged with two-third partial hepatectomy at 28 days and 70 days after birth to determine the regenerative capacity of their livers. As a result, it was found that iPS-hepatocytes responded to the hepatectomy with characteristics of normal liver regeneration. The results reported from this study clearly indicate that iPS cells are able to differentiate into mature and functional hepatocytes *in vivo* following the embryonic hepatocyte-differentiation program, and thus provide a practical and experimental basis and rationale to generate hu-iPS-hepatocyte-*h*-chimeric mice for drug testing and HCV/HBV-related studies. To this end, it is necessary to create an alternative method in which hu-iPS cells can be incorporated into the m-liver as normally differentiated hepatocytes, which does not include a procedure of hu-iPS cell-injection into the host blastocysts. It is also possible to create a hepatocyte-*h*-m-model by transplanting hu-iPS cell-derived hepatocytes into the livers of young immunodeficient and liver-injured mice, such as the uPA/SCID mouse. To the authors' knowledge, studies are actively being undertaken to achieve this goal, although no trial has been reported in which sufficiently high levels of RI of the donor cells were attained.

7. Expert opinion

The time 'distance' from the discovery of a candidate chemical to the creation of a clinical drug is becoming shorter, and the risk of failing to produce a reliable and effective medicine is decreasing as knowledge of science and technology increases and deepens. The hepatocyte-*h*-m-model is one example of such relations between principles and applications in the world of science and technology. Before the generation of the hepatocyte-*h*-m-model, there was a complete separation between R&D (preclinical) and clinical tests, which were mostly conducted with animal-derived or human-derived systems in laboratories and with completely hu-systems in hospitals, respectively. Since the generation of the hepatocyte-*h*-m-model, a living experimental model of hu-liver functions, this clear separation has become diffuse and obscure due to the involvement of hu-liver-like functions in the m-liver. In this overview, the current status of the hepatocyte-*h*-mouse is described, including its usability for drug testing, such as predicting metabolic profiles and PK in humans and studies of the mechanisms of hepatitis viruses. Accumulating data from these studies indicate that the chimeric mouse is largely faithful in reproducing metabolic profiles of drugs and their kinetics in humans, especially those that are processed through CYP 450 enzymes if their RIs are high (> ~ 70%).

Although there is still much work to do for improving the currently available hepatocyte-*h*-mice to increase their

usability, it is thought that the basic technologies have been established and as described in this review, the first-generation hepatocyte-*h*-mice practically meets the requirements from laboratories for drug development and for researches of hepatitis viruses. Therefore, the researchers' interests are now moving from the first-generation chimeric mouse model to the second-generation chimeric model. Challenges have been robustly undertaken for improving hepatocyte-*h*-mice (first-generation chimeric mice) and overcoming their shortcomings, such as the absence of hu-immune cells required for inducing inflammations and immunological reactions. These innovative research trials have been creating second-generation chimeric mice with dual chimerism (hepatocytes and inflammation/immune-response cells), with which researchers could examine drug- or hepatitis virus-induced inflammation and hepatic fibrogenesis. Hopefully, in the near future, researchers could generate the second-generation *h*-mice that develop hepatocytic carcinogenesis under appropriate experimental conditions. Modern progressions in biotechnology are also making large contributions to the usability and availability of animal models to understand drug metabolism, toxicity and effectiveness, which includes the creation of liver-*h* rats and generation of chimeric mice with 'artificial hu-hepatocytes' using the patients' fibroblasts and iPS cell technology.

Acknowledgments

The authors acknowledge the studies that were undertaken which have investigated drug metabolism in chimeric mouse livers and hepatitis virus infections led by Professors Y Yokoi of Kanazawa University and K Chayama of Hiroshima University, respectively. Furthermore, the authors express their appreciation to S Sugai for providing them practical information about the success rate of candidate compounds that survive for a sufficient periods of time as drugs for public use. The authors acknowledge permission of reproduction of the Figures from the Nature Publishing Group.

Declaration of interest

K Yoshizato and C Tateno are employees of PhoenixBio Co., Ltd.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Hyodo K, Yamamoto E, Suzuki T, et al. Development of liposomal anticancer drugs. Biol Pharm Bull 2013;36(5):703-7
2. Sun H, Xia M, Austin CP, Huang R. Paradigm shift in toxicity testing and modeling. AAPS J 2012;14(3):473-80
3. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 2004;3:711-15
4. Dr. Sugai, S in personal communication
5. Renaud HJ, Cui JY, Khan M, Klaassen CD. Tissue distribution and gender-divergent expression of 78 cytochrome P450 mRNAs in mice. Toxicol Sci 2011;124:261-77
6. Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med 2005;352:2211-21; Review
7. Jiang XL, Gonzalez FJ, Yu AM. Drug-metabolizing enzyme, transporter, and nuclear receptor genetically modified mouse models. Drug Metab Rev 2011;43:27-40
8. Liggett SB. Genetically modified mouse models for pharmacogenomic research. Nat Rev Genet 2004;5:657-63; Review
9. Muruganandan S, Sinal CJ. Mice as clinically relevant models for the study of cytochrome P450-dependent metabolism. Clin Pharmacol Ther 2008;83:818-28
   • **This review is useful for readers who are interested in comparatively understanding the two animal models of drug testing: 'gene-humanized' and 'hepatocyte-humanized mice'.**
10. Dandri M, Burda MR, Török E, et al. Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology 2001;33:981-8
11. Mercer DF, Schiller DE, Elliott JF, et al. Hepatitis C virus replication in mice with chimeric human livers. Nat Med 2001;7:927-33
12. Tateno C, Yoshizane Y, Saito N, et al. Near completely humanized liver in mice shows human-type metabolic responses to drugs. Am J Pathol 2004;165:901-12
13. Yoshizato K, Tateno C. In vivo modeling of human liver for pharmacological study using humanized mouse. Expert Opin Drug Metab Toxicol 2009;5:1435-46; Review
14. Yoshizato K, Tateno C, Utoh R. The mechanism of liver size control in mammals: a novel animal study. Int J Des Nature Ecodynamics 2009;4:123-42
15. Yoshizato K, Tateno C. A human hepatocyte-bearing mouse: an animal model to predict drug metabolism and effectiveness in humans. PPAR Res 2009;2009:476217
16. Yoshizato K, Tateno C, Utoh R. Mice with liver composed of human hepatocytes as an animal model for drug testing. Curr Drug Discov Technol 2012;9(1):63-76
17. Sandgren EP, Degen JL, Palmiter RD, et al. Replacement of diseased mouse liver by hepatic cell transplantation. Science 1994;263:1149-52
   • **This paper described the original idea and methodology that later led to the generation of hepatocyte-humanized mice.**
18. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature 1983;301:527-30
19. Schatz DG. Antigen receptor genes and the evolution of a recombinase. Semin Immunol 2004;16:245-56
20. Azuma H, Paulk N, Ranade A, et al. Robust expansion of human hepatocytes in Fah(-/-)/Rag2(-/-)/Il2rg(-/-) mice. Nat Biotechnol 2007;25:903-10
21. Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood

cell-transplanted mice. Science 2004;304:104-7

22. Gorantla S, Sneller H, Walters L, et al. Human immunodeficiency virus type 1 pathobiology studied in humanized BALB/c-Rag2-/-gammac-/- mice. J Virol 2007;81:2700-12

23. Shinkai Y, Rathbun G, Lam KP, et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell 1992;68:855-67

24. Perryman LE. Molecular pathology of severe combined immunodeficiency in mice, horses, and dogs. Vet Pathol 2004;41:95-100

25. Bosma MJ, Carroll AM. The SCID mouse mutant: definition, characterization, and potential uses. Annu Rev Immunol 1991;9:323-50; Review

26. Suemizu H, Hasegawa M, Kawai K, et al. Establishment of a humanized model of liver using NOD/Shi-scid IL2Rgnull mice. Biochem Biophys Res Commun 2008;377:248-52

27. Kikutani H, Makino S. The murine autoimmune diabetes model: NOD and related strains. Adv Immunol 1992;51:285-322

28. Ohbo K, Suda T, Hashiyama M, et al. Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor gamma chain. Blood 1996;87:956-67

29. Heckel JL, Sandgren EP, Degc JL, et al. Neonatal bleeding in transgenic mice expressing urokinase-type plasminogen activator. Cell 1990;62:447-56

30. Mohammed FF, Pennington CJ, Kassiri Z, et al. Metalloproteinase inhibitor TIMP-1 affects hepatocyte cell cycle via HGF activation in murine liver regeneration. Hepatology 2005;41:857-67

31. Bissig KD, Le TT, Woods NB, Verma IM. Repopulation of adult and neonatal mice with human hepatocytes: a chimeric animal model. Proc Natl Acad Sci USA 2007;104:20507-11

32. Grompe M, et al. Loss of fumarylacetoacetate hydrolase is responsible for the neonatal hepatic dysfunction phenotype of lethal albino mice. Genes Dev 1993;7:2298-307

33. Brezillon NM, DaSilva L, L'Hôte D, et al. Rescue of fertility in homozygous mice for the urokinase plasminogen activator transgene by the transplantation of mouse hepatocytes. Cell Transplant 2008;17:803-12

34. Sandgren EP, Palmiter RD, Heckel JL, et al. Complete hepatic regeneration after somatic deletion of an albumin-plasminogen activator transgene. Cell 1991;66:245-56

35. Okamura K, Asahina K, Fujimori H, et al. Generation of hybrid hepatocytes by cell fusion from monkey embryoid body cells in the injured mouse liver. Histochem Cell Biol 2006;125:247-57

36. Tateno C, Miya F, Wake K, et al. Morphological and microarray analyses of human hepatocytes from xenogeneic host livers. Lab Invest 2013;93(1):54-71

37. Lootens L, Meuleman P, Pozo OJ, et al. uPA+/+-SCID mouse with humanized liver as a model for in vivo metabolism of exogenous steroids: methandienone as a case study. Clin Chem 2009;55:1783-93

38. Okumura H, Katoh M, Sawada T, et al. Humanization of excretory pathway in chimeric mice with humanized liver. Toxicol Sci 2007;97:533-8

39. Botrè F. Humanized animal models to study drug metabolism: no longer a "chimera"? Clin Chem 2009;55(10):1763-4

40. Lootens L, Van Eenoo P, Meuleman P, et al. The uPA(+/+)-SCID mouse with humanized liver as a model for in vivo metabolism of 4-androstene-3,17-dione. Drug Metab Dispos 2009;37:2367-74

41. Dai Y, Su W, Ding Z, et al. Mehta JL. Regulation of MSR-1 and CD36 in macrophages by LOX-1 mediated through PPAR-γ. Biochem Biophys Res Commun 2013; [Epub ahead of print]

42. Schulz-Utermoehl T, Sarda S, Foster JR, et al. Evaluation of the pharmacokinetics, biotransformation and hepatic transporter effects of troglitazone in mice with humanized livers. Xenobiotica 2012;42(6):503-17

43. Sanoh S, Horiguchi A, Sugihara K, et al. Predictability of metabolism of ibuprofen and naproxen using chimeric mice with human hepatocytes. Drug Metab Dispos 2012;40(12):2267-72

44. Sanoh S, Horiguchi A, Sugihara K, et al. Prediction of in vivo hepatic clearance and half-life of drug candidates in human using chimeric mice with humanized liver. Drug Metab Dispos 2012;40(2):322-8

45. Garattini E, Mendel R, Romão MJ, et al. Mammalian molybdo-flavoenzymes, an expanding family of proteins: structure, genetics, regulation, function and pathophysiology. Biochem J 2003;372(Pt 1):15-32; Review

46. Sanoh S, Nozaki K, Murai H, et al. Prediction of human metabolism of FK3453 by aldehyde oxidase using chimeric mice transplanted with human or rat hepatocytes. Drug Metab Dispos 2012;40(1):76-82

47. Pryde DC, Dalvie D, Hu Q, et al. Aldehyde oxidase: an enzyme of emerging importance in drug discovery. J Med Chem 2010;53:8441-60

48. Garattini E, Terao M. Increasing recognition of the importance of aldehyde oxidase in drug development and discovery. Drug Metab Rev 2011;43:374-86

49. Akabane T, Tanaka K, Irie M, et al. Case report of extensive metabolism by aldehyde oxidase in humans: pharmacokinetics and metabolite profile of FK3453 in rats, dogs, and humans. Xenobiotica 2011;41:372-84

50. Brunner C, Muller B, Wirth T. Bruton's tyrosine kinase is involved in innate and adaptive immunity. Histol Histopathol 2005;20:945-55

51. Pan Z. Bruton's tyrosine kinase as a drug discovery target. Drug News Perspect 2008;21:357-62

52. Liu L, Di Paolo J, Barbosa J, et al. Antiarthritis effect of a novel Bruton's tyrosine kinase (BTK) inhibitor in rat collagen-induced arthritis and mechanism based pharmacokinetic/pharmacodynamic modeling: relationships between inhibition of BTK phosphorylation and efficacy. J Pharmacol Exp Ther 2011;338:154-63

53. Liu L, Halladay JS, Shin Y, et al. Significant species difference in amide hydrolysis of GDC-0834, a novel potent and selective Bruton's tyrosine kinase inhibitor. Drug Metab Dispos 2011;39(10):1840-9

54. Ploss A, Rice CM. Towards a small animal model for hepatitis C. EMBO Rep 2009;10:1220-7

55. Uprichard SL, Chung J, Chisari FV, Wakita T. Replication of a hepatitis C

virus replicon clone in mouse cells. Virol J 2006;3:89

56. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 2005;11:791-6

57. Bissig KD, Wieland SF, Tran P, et al. Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. J Clin Invest 2010;120:924-30

58. Tsuge M, Hiraga N, Takaishi H, et al. Yoshizato K. nfection of human hepatocyte chimeric mouse with genetically engineered hepatitis B virus. Hepatology 2005;42(5):1046-54

59. Kimura T, Imamura M, Hiraga N, et al. Establishment of an infectious genotype 1b hepatitis C virus clone in human hepatocyte chimeric mice. J Gen Virol 2008;89(Pt 9):2108-13

60. Kamiya N, Iwao E, Hiraga N, et al. Practical evaluation of a mouse with chimeric human liver model for hepatitis C virus infection using an NS3-4A protease inhibitor. J Gen Virol 2010;91(Pt 7):1668-77

61. Ohara E, Hiraga N, Imamura M, et al. Elimination of hepatitis C virus by short term NS3-4A and NS5B inhibitor combination therapy in human hepatocyte chimeric mice. J Hepatol 2011;54(5):872-8

62. Abe H, Imamura M, Hiraga N, et al. ME3738 enhances the effect of interferon and inhibits hepatitis C virus replication both in vitro and in vivo. J Hepatol 2011;55(1):11-18

63. Perni RB, Almquist SJ, Byrn RA, et al. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. Antimicrob Agents Chemother 2006;50:899-909

64. Hiraga N, Imamura M, Abe H, et al. Rapid emergence of telaprevir resistant hepatitis C virus strain from wildtype clone in vivo. Hepatology 2011;54(3):781-8

65. Song X, Guo Y, Duo S, et al. A mouse model of inducible liver injury caused by tet-on regulated urokinase for studies of hepatocyte transplantation. Am J Pathol 2009;175:1975-83

66. Held WA, Mullins JJ, Kuhn NJ, et al. T antigen expression and tumorigenesis in transgenic mice containing a mouse major urinary protein/SV40 T antigen hybrid gene. EMBO J 1989;8:183-91

67. Weglarz TC, Degen JL, Sandgren EP. Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes. Am J Pathol 2000;157:1963-74

68. He Z, Zhang H, Zhang X, et al. Liver xeno-repopulation with human hepatocytes in Fah-/-Rag2-/- mice after pharmacological immunosuppression. Am J Pathol 2010;177:1311-19

69. Escribano O, Fernández-Moreno MD, Piña MJ, et al. Pretreatment with FK506 up-regulates insulin receptors in regenerating rat liver. Hepatology 2002;36:555-61

70. Legrand N, Ploss A, Balling R, et al. Humanized mice for modeling human infectious disease: challenges, progress, and outlook. Cell Host Microbe 2009;6:5-9; Review

71. Bowen DG, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature 2005;436:946-52; Review

72. Washburn ML, Bility MT, Zhang L, et al. A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. Gastroenterology 2011;140:1334-44

•• **The authors put forward the idea and technology for the second-generation chimeric mice, that is, mice with dual chimerisms, hepatocytes and immune cells.**

73. Di Stasi A, Tey SK, Dotti G, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med 2011;365(18):1673-83

74. Pajvani UB, Trujillo ME, Combs TP, et al. Fat apoptosis through targeted activation of caspase 8: a new mouse model of inducible and reversible lipoatrophy. Nat Med 2005;11:797-803

75. Robinet E, Baumert TF. A first step towards a mouse model for hepatitis C virus infection containing a human immune system. J Hepatol 2011;55:718-20

76. Bataller R, Paik YH, Lindquist JN, et al. Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells. Gastroenterology 2004;126:529-40

77. Utoh R, Tateno C, Kataoka M, et al. Hepatic hyperplasia associated with discordant xenogeneic parenchymal-nonparenchymal interactions in human hepatocyte-repopulated mice. Am J Pathol 2010;177:654-65

78. Tsuge M, Fujimoto Y, Hiraga N, et al. Hepatitis C virus infection suppresses the interferon response in the liver of the human hepatocyte chimeric mouse. PLoS ONE 2011;6(8):e23856

79. Okazaki A, Hiraga N, Imamura M, et al. Severe necroinflammatory reaction caused by natural killer cell-mediated Fas/Fas ligand interaction and dendritic cells in human hepatocyte chimeric mouse. Hepatology 2012;56:555-66

80. Turrini P, Monego G, Gonzalez J, et al. Human hepatocytes in mice receiving]g pre-immune injection with human cord blood cells. Biochem Biophys Res Commun 2005;326(1):66-73

81. Cruse JM, Lewis RE Jr. The immune system victorious: selective preservation of self. Immunol Res 1993;12(2):101-14; Review

82. Porteus MH, Carroll D. Gene targeting using zinc finger nucleases. Nat Biotechnol 2005;23(8):967-73

83. Geurts AM, Cost GJ, Freyvert Y, et al. Knockout rats via embryo microinjection of zinc-finger nucleases. Science 2009;325(5939):433

84. Mashimo T, Takizawa A, Kobayashi J, et al. Generation and characterization of severe combined immunodeficiency rats. Cell Rep 2012;2(3):685-94

85. Katayama S, Tateno C, Asahara T, Yoshizato K. Size-dependent in vivo growth potential of adult rat hepatocytes. Am J Pathol 2001;158(1):97-105

86. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126(4):663-76

87. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318(5858):1917-20

88. Si-Tayeb K, Noto FK, Nagaoka M, et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 2010;51(1):297-305

89. Takayama K, Inamura M, Kawabata K, et al. Generation of metabolically functioning hepatocytes from human pluripotent stem cells by FOXA2 and HNF1alpha transduction. J Hepatol 2012;57(3):628-36

90. Schwartz RE, Trehan K, Andrus L, et al. Modeling hepatitis C virus infection using human induced pluripotent stem cells. Proc Natl Acad Sci USA 2012;109(7):2544-8

•• **This paper demonstrated that iPS cell-derived hu-hepatocyte-like cells are permissive to HCV infection in culture and suggests their usefulness as a faithful in vitro modeling of HCV-induced hepatic diseases.**

91. Wu X, Robotham JM, Lee E, et al. Productive hepatitis C virus infection of stem cell-derived hepatocytes reveals a critical transition to viral permissiveness during differentiation. PLoS Pathog 2012;8(4):e1002617

92. Marukian S, Andrus L, Sheahan TP, et al. Hepatitis C virus induces interferon-λ and interferon-stimulated genes in primary liver cultures. Hepatology 2011;54(6):1913-23

93. Espejel S, Roll GR, McLaughlin KJ, et al. Induced pluripotent stem cell-derived hepatocytes have the functional and proliferative capabilities needed for liver regeneration in mice. J Clin Invest 2010;120(9):3120-6

94. Grompe M, Lindstedt S, al-Dhalimy M, et al. Pharmacological correction of neonatal lethal hepatic dysfunction in a murine model of hereditary tyrosinaemia type I. Nat Genet 1995;10(4):453-60

Affiliation

Katsutoshi Yoshizato[†] & Chise Tateno
[†]Author for correspondence
Professor Emeritus, Academic Adviser,
Phoenixbio - Academic Adviser Office,
3-4-1, Kagamiyama, Higashihiroshima,
Hiroshima 739-0016, Japan
E-mail: katsutoshi.yoshizato@phoenixbio.co.jp

Xenotransplantation 2013; 20: 227–238
Printed in Singapore. All rights reserved
doi: 10.1111/xen.12037

Original Article

Repopulation of the immunosuppressed retrorsine-treated infant rat liver with human hepatocytes

Tachibana A, Tateno C, Yoshizato K. Repopulation of the immunosuppressed retrorsine-treated infant rat liver with human hepatocytes. Xenotransplantation 2013: 20: 227–238. © 2013 John Wiley & Sons A/S.

Abstract: Background: We previously generated humanized chimeric mice by transplanting h-hepatocytes into the livers of the diseased-liver transgenic mouse model with immunodeficient background. These mice with livers mostly replaced by human (h) hepatocytes have been proved to be useful for research on drug metabolism and toxicity and on intrahepatic pathogens such as hepatitis. However, their small body size prohibited collecting sufficient biological samples and made surgical manipulation difficult, which motivated us to produce humanized larger animal(s) bearing h-hepatocytes.
Methods: Fischer 344 (F344) rats at 2 weeks of age were administrated with hepatotoxin retrorsine (RS) and then transplanted with syngeneic F344 rat (r)- or h-hepatocytes via the portal vein. The hosts were injected daily with FK506 immunosuppressant. The livers were harvested periodically for determining donor-cell replacement ratios and compared with those of the humanized chimeric mice, and liver-specific mRNA and protein expressions by immunohistochemistry and reverse-transcription PCR.
Results: RS treatment of infant rats inhibited hepatocyte proliferation, resulting in decreased liver weight and megalocytic changes in hepatocytes. R-hepatocytes transplanted into RS-treated rats engrafted into and repopulated the liver at ratios of 16.4 ± 6.7% and 48.3 ± 29.3% at 3 and 6 weeks after transplantation, respectively. H-hepatocytes also engrafted into the rat liver and showed a repopulation ratio of 2.5 ± 1.5% at 3 weeks post-transplantation, which was comparable to the ratio in the humanized chimeric mouse model at least until 3 weeks. Propagated h-hepatocytes in the rat liver expressed hepatocyte-specific mRNA and proteins at least 3 weeks after transplantation.
Conclusions: Xenogeneic hepatocytes were able to engraft rat liver and grow well therein for at least 3 weeks post-transplantation in rats when immunosuppression was combined appropriately with liver injury at comparable levels to the well-characterized humanized chimeric mouse model.

Asato Tachibana,[1,2] Chise Tateno[1,2,3] and Katsutoshi Yoshizato[1,2,3,4,†]

[1]Yoshizato Project, Cooperative Link of Unique Science and Technology for Economy Revitalization (CLUSTER), Hiroshima Prefectural Institute of Industrial Science and Technology, Higashihiroshima, Hiroshima, [2]PhoenixBio Co. Ltd, Higashihiroshima, Hiroshima, [3]Hiroshima University Liver Research Project Center, Hiroshima, Hiroshima, [4]Developmental Biology Laboratory and Hiroshima University 21st Century COE Program for Advanced Radiation Casualty Medicine, Department of Biological Science, Graduate School of Science, Hiroshima University, Higashihiroshima, Hiroshima, Japan
* Present address Synthetic Biology Laboratory Center, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-Ku, Osaka, 545-8585, Japan

Key words: human hepatocytes – retrorsine – xenotransplantation

Abbreviations: Alb, albumin; AAT, α1-antitrypsin; BMCs, bone marrow cells; BrdU, bromodeoxyuridine; CYP, cytochrome P450; CK, cytokeratin; DPPIV, dipeptidyl dipeptidase-IV; F344, fischer 344; G6P, glucose-6-phosphatase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBV, hepatitis B virus; HCV, hepatitis C virus; HNF-4, hepatocyte nuclear factor-4; h, human; PH, partial hepatectomy; r, rat; r/h, rat and human; RI, replacement index; RS, retrorsine; RT, reverse transcription; SCID, severe combined immunodeficiency; uPA, urokinase-type plasminogen activator

Address reprint requests to Katsutoshi Yoshizato and Chise Tateno, PhoenixBio. Co. Ltd., 3-4-1 Kagamiyama, Higashishiroshima, Hiroshima 739-0046, Japan. (E-mails: katsutoshi.yoshizato@phoenixbio.co.jp and chise.mukaidani@phoenixbio.co.jp)

Received 16 August 2012;
Accepted 9 April 2013

Introduction

Hepatocytes play a major role in a variety of metabolic processes mediated by the reactions of many kinds of enzymes. Because humans and animals differ in isoform composition, expression levels, and catalytic activities of drug-metabolizing enzymes, human (h) hepatocytes under in vitro